RIECKER MEMORIAL LIBRARY
ST. JOSEPH MERCY HOSPITAL
ANN ARBOR, MI 48106

1982

cancer treatment reports

November 1982
Volume 66
Number 11
Pages 1889-2004

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

cancer treatment reports

National Cancer Institute, Vincent T. DeVita, Jr., ***Director***
Division of Cancer Treatment, Bruce A. Chabner, ***Director***
Scientific Information Branch, Susan Molloy Hubbard, ***Chief***

Editorial Board

John S. Macdonald, ***Editor-In-Chief***

Associate Editors

Martin D. Abeloff
Joseph Aisner
Lewis Aronow
Richard L. Cysyk
Ross C. Donehower
William D. De Wys
Daniel C. Ihde
Allen S. Lichter
Robert W. Makuch
David P. Pistenmaa
Philip A. Pizzo
Charles A. Schiffer
Mary K. Wolpert

Advisory Board

Gianni Bonadonna
George P. Canellos
Richard H. Creech
Harvey M. Golomb
Robert L. Goodman
Stephen B. Howell
Randall K. Johnson
Irwin H. Krakoff
Brigid G. Leventhal
Robert B. Livingston
Albert F. LoBuglio
William L. McGuire
Franco M. Muggia
Carol S. Portlock
Sydney E. Salmon

Editorial Staff

Jean Griffin Baum, ***Managing Editor***
Dee Kittner LeRoy, ***Assistant Managing Editor***

Editors

Elaine Price Beck
Anne McCarthy
Marian Sharp McGrath

Editorial Assistants

Elaine Brown
Susan Lee Gordon
Jana Johnston
Barbara Lineberry

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports*. Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 7910 Woodmont Ave, Rm 8C08, Landow Bldg, Bethesda, MD 20814 (301-496-6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors," which appears in the second page of every issue.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.

INSTRUCTIONS TO AUTHORS

Title Page
The complete title of the manuscript and the full name, affiliation, and address of each author should be included on the title page. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary
Begin the article with a short summary.

Text
Submit an original and 3 copies of the manuscript, typed double-spaced. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, title of book, edition (if more than one), city and name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers presented at meetings should include author; title of paper; title, city, and date of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print. Color photographs cannot be used.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names or The Merck Index* for proper nomenclature.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Summaries
Manuscripts published in this section can be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. A second table will be permitted only when requested by the editorial board. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints
Supplied free of charge: 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior author about 3 weeks after publication of the article.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

Contents

Cancer Treatment Reports
Volume 66, Number 11
November 1982

Special Feature

1889 Influence of Diuretics on Urinary General Base Catalytic Activity and Cyclophosphamide-Induced Bladder Toxicity. **N. E. Sladek, P. C. Smith, P. M. Bratt, J. E. Low, J. F. Powers, R. F. Borch, and J. R. Coveney.** University of Minnesota, Minneapolis

1901 Editorial on Management of Drug Toxicity. **P. V. Woolley.** Georgetown University Medical Center, Vincent T. Lombardi Cancer Research Center, Washington, DC

Clinical and Phase I–II Studies

1903 Intracavitary Bleomycin in the Management of Malignant Effusions: A Multicenter Study. **M. J. Ostrowski and G. M. Halsall.** Norfolk and Norwich Hospital, Norwich, England

1909 Hydroxy-9-methyl-2-ellipticinium for Osseous Metastases From Breast Cancer: A 5-Year Experience. **P. Juret, J. F. Heron, J. E. Couette, T. Delozier, and J. Y. Le Talaer.** Centre François Baclesse, Caen, France

1917 Results of Aggressive Chemotherapy for Acute Nonlymphoblastic Leukemia in Adults. **J. O. Armitage, C. P. Burns, F. R. Dick, S. B. Aunan, and D. J. Slymen.** University of Iowa College of Medicine, Iowa City

1925 Use of Methotrexate and 5-FU for Recurrent Head and Neck Cancer. **C. Jacobs.** Stanford University School of Medicine, California

1929 Phase II Trial of Mitoxantrone. **K. C. Anderson, G. I. Cohen, and M. B. Garnick.** Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

Clinical and Preclinical Pharmacology

1933 Cisplatin Urinary Pharmacokinetics and Nephrotoxicity: A Common Circadian Mechanism. **F. Levi, W. J. M. Hrushesky, R. F. Borch, M. E. Pleasants, B. J. Kennedy, and F. Halberg.** University of Minnesota Medical School, Minneapolis

1939 Toxicology of AMSA in Beagle Dogs and CDF$_1$ Mice. **K. L. Pavkov and D. C. Thake.** Battelle, Columbus Laboratories, Ohio

1945 Activity of 1-*p*-(3,3-Dimethyl-1-triazeno) Benzoic Acid Potassium Salt in M 5076/73A Ovarian Reticular Cell Sarcoma of the Mouse. **T. Colombo, S. Garattini, L. Lassiani, and M. D'Incalci.** Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy

1949 Hematopoietic Stem Cells in Friend Murine Leukemia Virus-Infected Mice Undergoing Chemotherapy: Effects of 5-FU. **H. J. Seidel and L. Kreja.** University of Ulm, W Germany

1957 Studies of the Pharmacology of *N*-Methylformamide in Mice. **C. Brindley, A. Gescher, E. S. Harpur, D. Ross, J. A. Slack, M. D. Threadgill, and H. Whitby.** CRC Experimental Chemotherapy Research Group, University of Aston,Birmingham, England

BRIEF REPORTS

1967 Massive Vincristine Overdose: Failure of Leucovorin To Reduce Toxicity. **L. T. Thomas, P. C. Braat, R. Somers, and R. Goudsmit.** University Hospital, Whilhelmina Gasthuis, University of Amsterdam, The Netherlands

1971 Combination Chemotherapy for Multiple Myeloma With Melphalan, Prednisone, Cyclophosphamide, Vincristine, and Carmustine (BCNU) (M-2 Protocol). **U. Tirelli, D. Crivellari, A. Carbone, A.**

Veronesi, E. Galligioni, M. G. Trovò, S. Tumolo, and E. Grigoletto. General Hospital, Pordenone, Italy

1975 Results of a Randomized Double-Blind Crossover Trial of Scopolamine Versus Placebo Administered by Transdermal Patch for the Control of Cisplatin-Induced Emesis. **D. L. Longo, M. Wesley, D. Howser, S. M. Hubbard, T. Anderson, and R. C. Young.** National Cancer Institute, Bethesda, Maryland

1977 Salvage Chemotherapy for Non-Hodgkin's Lymphoma. **R. P. Ng, D. Todd, and R. K. K. Khoo.** Queen Mary Hospital, Hong Kong

1981 Ip Immunotherapy and Chemotherapy in Advanced Epithelial Ovarian Cancer. **E. Hernandez, N. B. Rosenshein, J. Pino y Torres, J. Villar, F. C. Grumbine, D. S. Ettinger, M. B. Dillon, J. L. Klein, and S. E. Order.** The Johns Hopkins Medical Institutions, Baltimore, Maryland

1983 Autologous Bone Marrow Transplantation in Acute Myeloid Leukemia in Relapse or in Complete Remission. **P. Herve, A. Rozenbaum, E. Plouvier, M. Flesch, J. Y. Cahn, A. Farradji, J. P. Bergerat, J. C. Adjizian, and B. Pignon.** Centre Régional de Transfusion Sanguine, Besançon, France

1987 Multiple Squamous Cell Carcinoma Treated With Intra-arterial Cisplatin in a Patient With Rheumatoid Arthritis. **J. A. Ajani and M. A. Burgess.** University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

CLINICAL TRIALS SUMMARIES

1991 High-Dose AMSA and Autologous Bone Marrow Transplantation in Relapsed Acute Leukemia. **A. R. Zander, L. Vellekoop, K. A. Dicke, M. J. Keating, K. B. McCredie, S. S. Legha, G. Spitzer, D. Verma, and G. P. Bodey.** University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

1993 *m*-AMSA in Disseminated Prostatic Carcinoma: A Phase II Study. **A. Drelichman, D. A. Decker, M. Al-Sarraf, and C. B. Dhabuwala.** Wayne State University, School of Medicine, Detroit, Michigan

1995 Phase II Trial of Mitoxantrone in Advanced Colorectal Cancer. **E. M. Bonnem, E. P. Mitchell, P. V. Woolley, F. P. Smith, J. Neefe, L. Smith, and P. S. Schein.** Georgetown University Hospital and Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC

1997 Phase II Trial of Bisantrene in Advanced Colorectal Cancer: A Cancer and Leukemia Group B Study. **M. C. Perry, A. A. Forastiere, F. Richards, II, R. B. Weiss, and D. Anbar.** Cancer and Leukemia Group B

LETTERS

1999 VAP-Cyclo: A Short Intensive Regimen of Chemotherapy for Advanced Breast Cancer. **V. H. C. Bramwell, A. Howell, H. Scarffe, D. Crowther, and R. A. Sellwood.** Manchester University, Christie Hospital and Holt Radium Institute, England

2000 Subcutaneous Reaction Due to Intra-arterial Infusion of Doxorubicin. **A. Ballin, G. Kende, B. Morag, and M. Ronen.** Chaim Sheba Medical Center, Tel-Hashomer, Israel

2003 ANNOUNCEMENTS

RIECKER MEMORIAL LIBRARY
ST. JOSEPH MERCY HOSPITAL
ANN ARBOR, MI 48106

Influence of Diuretics on Urinary General Base Catalytic Activity and Cyclophosphamide-Induced Bladder Toxicity[1,2,3]

N. E. Sladek,* P. C. Smith, P. M. Bratt, J. E. Low, J. F. Powers, R. F. Borch, and J. R. Coveney[4]

The influence of diuretics on the induction of bladder toxicity by cyclophosphamide was investigated in rats. Following ip administration, about 3.5% of the cyclophosphamide was excreted as 4-hydroxycyclophosphamide. This amount was found to be compatible with the view that the urotoxic effects of cyclophosphamide are caused by the acrolein generated in the urine from 4-hydroxycyclophosphamide, the primary metabolite of cyclophosphamide. In situ, acrolein was more potent than 4-hydroperoxycyclophosphamide with regard to producing an increase in bladder weight; phosphoramide mustard was essentially without urotoxic activity. The urotoxic potency of 4-hydroperoxycyclophosphamide, but not that of acrolein, increased as the pH and/or the phosphate concentration of the infusion medium increased. This was as expected in view of the knowledge that release of acrolein from 4-hydroxycyclophosphamide or 4-hydroperoxycyclophosphamide is facilitated by the presence of general base catalysts, eg, phosphate and bicarbonate, and that the rate at which this reaction proceeds in the presence of these catalysts increases as their concentration and the pH increases. In vivo, diuretics that acidified the urine, eg, ammonium chloride and furosemide, prevented the increase in bladder weight ordinarily elicited by the dose of cyclophosphamide used in these experiments. In contrast, a diuretic, acetazolamide, that markedly increased urinary bicarbonate concentration and alkalinized the urine, did not. None of the diuretics altered the systemic metabolism and urinary excretion of cyclophosphamide nor did they alter the systemic action, as judged by spleen weight, of cyclophosphamide. These observations demonstrate that the pH of the urine and the urinary concentration of general base catalysts greatly influence the urotoxic potential of oxazaphosphorines such as cyclophosphamide. They indicate that while the use of acidifying diuretics is likely to be beneficial in minimizing oxazaphosphorine-induced bladder toxicity, the use of alkalinizing diuretics may not be helpful. [Cancer Treat Rep 66:1889–1900, 1982]

The clinical usefulness of the oxazaphosphorines cyclophosphamide and ifosfamide is frequently limited by their urotoxic effects, ie, hemorrhagic cystitis. This toxicity is effected largely by the acrolein released in the urine from 4-hydroxycyclophosphamide and 4-hydroxyifosfamide, the primary metabolites of cyclophosphamide and ifosfamide, respectively (1–3). Release of acrolein is facilitated by the presence of general base catalysts, eg, phosphate and bicarbonate; the rate of release increases as their concentration and/or pH increases [(4,5); J. E. Low and N. E. Sladek, unpublished observations].[5]

[1]Received Feb 8, 1982; revised June 3, 1982; accepted June 16, 1982.

[2]Supported by Public Health Service grant CA-21737 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]This is the 11th in a series of papers on cyclophosphamide metabolism. Parts of this investigation appear in abstract form (Pharmacologist 22:175, 1980).

[4]Department of Pharmacology, University of Minnesota, Minneapolis.

[5]In previous communications (4,5), we interpreted our data as indicating that the mechanism by which phosphate (and bicarbonate) facilitated the conversion of 4-hydroxycyclophosphamide to acrolein and phosphoramide mustard was one of bifunctional catalysis. Recent, as yet unpublished, findings in our laboratory challenge this view and indicate that the mechanism is one of general base catalysis. Therefore, the term "general base catalysts" is used in this paper to refer to those agents, eg, phosphate and bicarbonate, that catalyze the conversion of 4-hydroxycyclophosphamide to acrolein and phosphoramide mustard.

*_Reprint requests to:_ Dr. N. E. Sladek, Department of Pharmacology, University of Minnesota, 3-260 Millard Hall, 435 Delaware St SE, Minneapolis, MN 55455.

Diuretics are often used to mitigate the urotoxic effects of these drugs. Their use is based on the rationale that they will effectively cause a dilution of the toxic material and decrease its contact time with the bladder. Some diuretics affect urinary bicarbonate and phosphate content; the pH of the urine may also be changed.

Little is known about the effects of diuretics on the systemic and extrasystemic actions of the oxazaphosphorines. The effects of several diuretics on cyclophosphamide metabolism and cyclophosphamide-induced increased bladder weight and spleen atrophy were determined in this investigation. Also determined were the urinary excretion of 4-hydroxycyclophosphamide following the ip administration of cyclophosphamide and the influence of urinary pH and general base catalyst concentration on the urotoxic potential of cyclophosphamide metabolites in situ.

MATERIALS AND METHODS

Animals.—Sprague-Dawley rats were purchased from the Bio-Lab Corp, White Bear Lake, MN. They were housed in metabolic cages and were fed Purina Laboratory Chow (Ralston Purina Co, St. Louis) ad libitum except during the 6-hr urine collection period when all food was removed. Water was available throughout, except where indicated in specific experiments.

Drugs. — Cyclophosphamide, 4-hydroperoxycyclophosphamide, and phosphoramide mustard· cyclohexylamine were supplied by Dr. W. A. Zygmunt, Mead Johnson and Co, Evansville, IN; Dr. Richard Borch, University of Minnesota, Minneapolis; and Dr. H. B. Wood, Jr, Drug Development Branch, 2ivision of Cancer Treatment, National Cancer Institute, Bethesda, MD; respectively. Furosemide (Lasix) and acetazolamide sodium (Diamox) were purchased from the University of Minnesota Hospital Pharmacy, Minneapolis. Acrolein and ammonium chloride were purchased from the Aldrich Chemical Co, Milwaukee; and Matheson, Coleman & Bell Manufacturing Chemists, Norwood, OH; respectively.

Drug administration.—Cyclophosphamide was dissolved in 0.9% NaCl solution and was injected ip in a volume equivalent to 3 ml/kg. Acetazolamide, ammonium chloride, and furosemide were dissolved in water and were given orally in a volume equivalent to 8.8 ml/kg. Doses and administration schedules are noted with each experiment. All of the drug solutions were administered immediately after preparation.

Cyclophosphamide assay.—The cyclophosphamide content of blood and urine obtained from treated rats was determined by a gas chromatography method described previously (6). Blood samples (140 μl) were collected from the tail vein into heparinized pipettes at 15, 30, 45, 60, 90, 120, and 150 mins when the systemic half-life of cyclophosphamide was determined. Values obtained at 30–120 mins inclusive were used to calculate the half-life of cyclophosphamide.

Assay for plasma 4-hydroxycyclophosphamide.—A fluorometric assay (7,8) was used to determine plasma concentrations of 4-hydroxycyclophosphamide. Blood samples (140 μl) were collected from the tail vein into heparinized pipettes. Following centrifugation, 50 μl of the resultant plasma was placed into a tube containing 25 μl of an aqueous 10% $Na_2WO_4 \cdot 2H_2O$ solution and 200 μl of an aqueous 0.01 N HCl solution. The contents were vortexed, 250 μl of an aqueous 12% $HClO_4$ solution was added, and the contents were vortexed again. Following centrifugation, 350 μl of the resultant supernatant was added to 200 μl of *m*-aminophenol (5 mg/ml) and hydroxylamine hydrochloride (6 mg/ml) dissolved in aqueous 3 N HCl solution. All of the previous steps were performed at approximately 4°C. The reaction mixture was then placed into a boiling water bath for 15 mins, after which it was cooled to room temperature in the dark. The concentration of the product, 7-hydroxyquinoline, was determined in an Aminco-Bowman J4-8950 spectrophotofluorometer. The excitation wavelength was 346 nm; the emission wavelength was 500 nm. Plasma samples, taken before cyclophosphamide was administered, were used to obtain blank values. 4-Hydroperoxycyclophosphamide was used as the standard. The lower limit of sensitivity was approximately 1 nmol of 4-hydroperoxycyclophosphamide/ml of plasma. The assay is relatively specific for acrolein and compounds (eg, 4-hydroperoxycyclophosphamide, 4-hydroxycyclophosphamide, and aldophosphamide) that yield acrolein. However, although acrolein placed in water gave rise to net relative fluorescent intensities comparable to those effected by equivalent concentrations of 4-hydroperoxycyclophosphamide, acrolein placed in blood gave rise to net relative fluorescent intensities that were < 1/100 of those obtained when equivalent concentrations of 4-hydroperoxycyclophosphamide were placed in blood; the disparity was apparently due to the highly reactive nature of acrolein. The presence of aldophosphamide in biologic fluids has never been conclusively demonstrated, probably because of its very short half-life (5,9).

Assay for urinary 4-hydroxycyclophosphamide.—Male Sprague-Dawley rats weighing 300–350 g were anesthetized with 100 mg/kg of Inactin ip. Following the start of anesthesia, the right jugular vein and the ureters were catheterized with PE-50 and PE-10 polyethylene tubing, respectively. Normal saline solution was infused into the jugular vein at a rate of 55 μl/min throughout the remainder of the experiment to ensure a constant urine flow of 11 ± 1 μl/min. Two hrs after the initiation of the normal saline infusion, 50 mg/kg of cyclophosphamide in normal saline solution was given iv in 0.5 ml over a 5-min period. Collection of the urine

was initiated 1 hr after the start of the normal saline infusion. It was collected for ten successive 30-min periods into tubes containing 10 ml of water; the tubes were in ice water throughout the collection period. Preliminary experiments established the stability of 4-hydroxycyclophosphamide for 30 mins under these conditions. The fluorometric assay described above was then used to determine urinary concentrations of 4-hydroxycyclophosphamide. As above, 50-μl samples were used for this purpose. Urine collected during the 1-hr period immediately before the injection of cyclophosphamide was used to obtain blank values; preliminary experiments established that these did not vary appreciably during the collection period. Authentic 4-hydroperoxycyclophosphamide was used as the standard. The lower limit of sensitivity was approximately 1 nmol of 4-hydroperoxycyclophosphamide/ml of urine.

Bladder infusion and assessment of urotoxicity.—Female Sprague-Dawley rats weighing 155–200 g were anesthetized with either 30 mg/kg of pentobarbital ip or pentobarbital and ketamine, each given ip at a dose of 15 mg/kg. Water was removed at least 12 hrs before initiation of anesthesia to minimize urine production. Following the start of anesthesia, PE-50 polyethylene tubing was passed into the bladder via the urethra, the bladder was flushed twice with 0.2 ml of normal saline solution, and the catheter was removed. A second catheter, the void volume of which was cleared with the appropriate drug solution, was passed into the bladder and secured with 3-0 silk thread. Acrolein, phosphoramide mustard, or 4-hydroperoxycyclophosphamide in sodium phosphate buffer solution was then infused into the bladder and allowed to remain for 1 hr. The infusion volume was 0.2 ml. At the end of this period, the drug was removed, the bladder was flushed twice with 0.2 ml of normal saline solution, and the catheter was removed. Bladders were removed 48 hrs later, cleaned of their contents, blotted dry, and weighed.

Other assays.—Bicarbonate and inorganic phosphate concentrations were determined by methods described previously (10,11).

Data analysis.—Values were submitted to computer-assisted linear regression analysis to obtain log-linear functions.

RESULTS

Cyclophosphamide-induced bladder toxicity is reflected by an increase in bladder weight although bladder wet weight can be used as a valid criterion of the urotoxic potency of the oxazaphosphorines only within a limited dose range (3). The relationship between dose and response is shown in figure 1. As presented, the data suggest that male rats are more sensitive to the urotoxic action of cyclophosphamide than are female rats. However, when bladder weights are expressed per total body weight, the apparent sex difference becomes less obvious (calculations not presented). The systemic effects of cyclophosphamide include a decrease in spleen weight. The relationship between dose and response is shown in figure 2.

Furosemide and NH_4Cl effected a diuresis, acidified the urine, and prevented the increase in bladder weight usually caused by the dose of cyclophosphamide used; the urinary concentrations of bicarbonate and phosphate were decreased or unchanged (table 1). In contrast, acetazolamide, an alkalinizing diuretic, did not prevent the cyclophosphamide-induced increase in bladder weight even though it effected a greater diuresis than did NH_4Cl; the phosphate concentration was slightly decreased and the bicarbonate concentration was markedly increased. Furosemide and NH_4Cl also prevented the gross hematuria induced by 100 mg/kg of cyclophosphamide; acetazolamide did not (data not presented). Ammonium acetate, at a dose equimolar to that of NH_4Cl, did not prevent cyclophosphamide-induced bladder toxicity nor did it alter urine pH and flow (data not presented). None of the diuretics altered the systemic action of cyclophosphamide as judged by their inability to prevent the loss of spleen weight (table 1).

Plasma concentrations of 4-hydroxycylophosphamide following the administration of 50 mg/kg of cyclophosphamide to control rats are presented in figure 3. 4-Hydroxycyclophosphamide is cleared from the plasma with an apparent half-life of 37 mins. The half-life for cyclophosphamide was virtually identical (table 2).

Consistent with the lack of an effect by any of the diuretics on the systemic action of cyclophosphamide are the observations that they did not alter the constants that define the metabolism, distribution, and excretion of cyclophosphamide and 4-hydroxycyclophosphamide (table 2).

The next series of experiments was conducted to establish that the concentration of general base catalysts in, and the pH of, the urine are important determinants with regard to the potential of cyclophosphamide to induce hemorrhagic cystitis and to offer an explanation for the inability of acetazolamide to reduce the bladder toxicity associated with cyclophosphamide administration even though urine flow is increased. Inorganic phosphate rather than bicarbonate was chosen as the general base catalyst to illustrate these points because of the relative ease with which its concentration can be maintained at a fixed value over a prolonged period of time.

Infusing bladders with acrolein effected a dose-dependent increase in bladder weight (fig 4). Bladder weights of 178 mg were obtained when 0.38 μmol of acrolein (1.9 mM) was infused. Similar observations have been reported (1,3). Administration of 50 and 90 mg/kg of cyclophosphamide ip to male (230–260 g) and female (170–200 g) rats, respectively, caused an identical increase in bladder weight (fig 1). The rate at which ad-

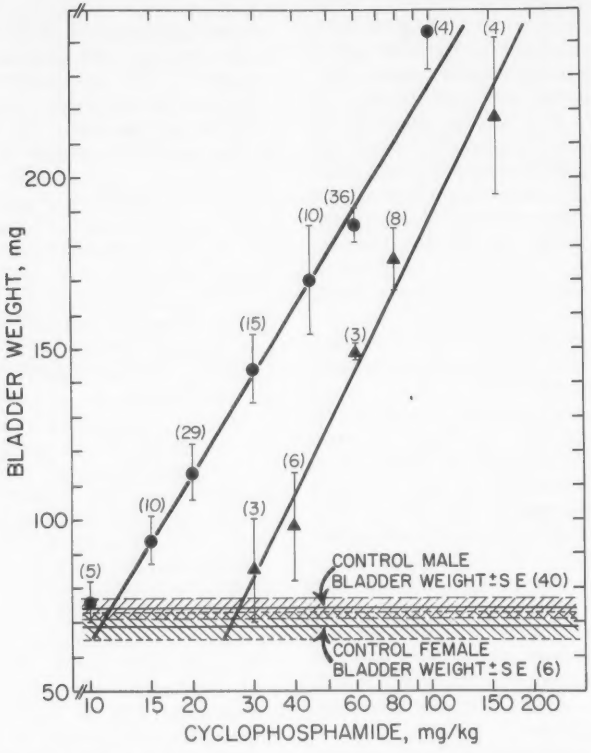


FIGURE 1.—Bladder weights in cyclophosphamide-treated rats: dose-response relationship. Male (●) and female (▲) Sprague-Dawley rats, 230–260 and 170–200 g, respectively, were injected ip with the doses of cyclophosphamide indicated. Bladder wet weights were determined 48 hrs later. Values are the mean ± SE; No. of animals is shown in parentheses.

TABLE 1.—Urinary and tissue weight changes in rats treated with cyclophosphamide and/or diuretics*

Treatment	Cyclophosphamide	Urine				Tissue weight (mg)	
		pH	Volume (ml)	PO_4 (mM)	HCO_3 (mEq/L)	Bladder	Spleen
H_2O	–	6.8 ± 0.1	3.4 ± 0.6	34 ± 5	7 ± 3	74 ± 3	572 ± 20
Acetazolamide	–	8.2 ± 0.2	10.6 ± 0.9	12 ± 2	133 ± 5	89 ± 7	578 ± 31
NH_4Cl	–	5.9 ± 0.1	7.3 ± 0.7	25 ± 4	1 ± 1	72 ± 5	684 ± 63
Furosemide	–	6.0 ± 0.1	18.7 ± 0.7	10 ± 2	3 ± 1	71 ± 3	545 ± 34
H_2O	+	6.8 ± 0.3	3.7 ± 0.7	32 ± 6	3 ± 1	186 ± 5	327 ± 12
Acetazolamide	+	8.1 ± 0.2	11.6 ± 0.8	14 ± 1	115 ± 4	168 ± 15	325 ± 11
NH_4Cl	+	5.8 ± 0.1	5.5 ± 0.2	30 ± 4	4 ± 1	79 ± 3	417 ± 13
Furosemide	+	6.1 ± 0.1	16.9 ± 5.2	14 ± 1	1 ± 1	93 ± 6	338 ± 30

*Male Sprague-Dawley rats, 230–260 g, were treated with the following diuretics before and/or after cyclophosphamide or 0.9% NaCl administration: 10 mg/kg of acetazolamide orally 15 mins before and 105 mins after, 660 mg/kg of NH_4Cl orally 120 mins before, or 88 mg/kg of furosemide orally 15 mins before. All diuretics were given as aqueous solutions; the volume given each time was 8.8 ml/kg. Animals that did not receive diuretics were given identical volumes of water. Values obtained for the 2 "H_2O" groups were pooled in each case because they were essentially identical. Cyclophosphamide was dissolved in 0.9% NaCl solution and 60 mg/kg was injected ip; the volume injected was 3 ml/kg. Animals that did not receive cyclophosphamide were injected with identical volumes of 0.9% NaCl solution. Urine was collected during the 6-hr period following cyclophosphamide injection; volume and pH of these collections were measured as were inorganic phosphate and bicarbonate concentrations. Bladder and spleen wet weights were determined 48 hrs after injection of cyclophosphamide. Values are the mean ± SE of measurements made on 3–18 animals.

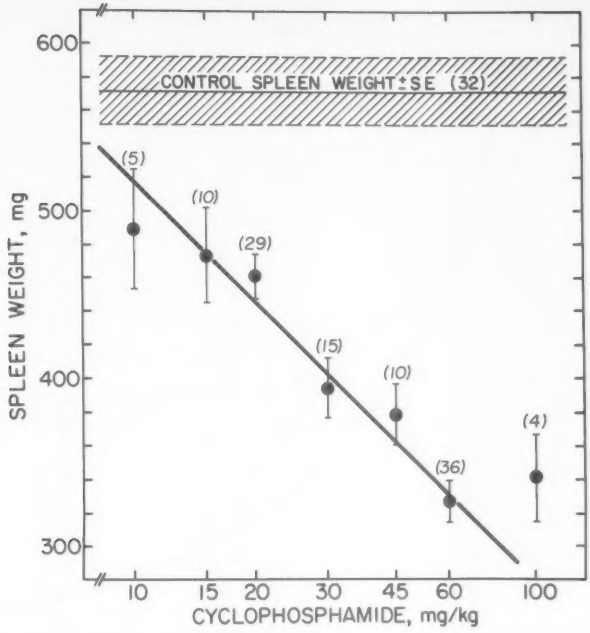


FIGURE 2.—Spleen weights in cyclophosphamide-treated rats: dose-response relationship. Male Sprague-Dawley rats, 230–260 g, were injected ip with the doses of cyclophosphamide indicated. Spleen wet weights were determined 48 hrs later. Values are the mean ± SE; No. of animals is shown in parentheses.

ducts between acrolein and sulfhydryls (ie, glutathione, acetylcysteine, or cysteine) are generated increases as the pH increases (12), suggesting the possibility of a similar relationship between acrolein and the critical target sites of the bladder. However, the increase in bladder weight effected by acrolein was essentially independent of the pH and phosphate concentration of the infusion medium (fig 4).

Infusing bladders with 4-hydroperoxycyclophosphamide also effected a dose-dependent increase in bladder weight (fig 5). 4-Hydroperoxycyclophosphamide was used in these experiments rather than 4-hy-

TABLE 2.—Half-lives of cyclophosphamide and 4-hydroxycyclophosphamide in rats treated with diuretics*

	Cyclophosphamide		4-Hydroxycyclophosphamide		
Diuretic	Half-life (mins)	% excreted in urine	Apparent half-life (mins)	Peak concentration (μM)	Area under curve (mM·min)
None	37 ± 4	8.3 ± 1.0	37 ± 6	27.6 ± 0.4	1.9 ± 0.1
Acetazolamide	35 ± 1	9.9 ± 0.5	42 ± 4	30.8 ± 2.1	2.0 ± 0.1
NH_4Cl	39 ± 4	6.9 ± 0.8	36 ± 4	30.5 ± 4.9	1.9 ± 0.2
Furosemide	33 ± 2	9.4 ± 1.9	44 ± 7	28.8 ± 1.6	1.9 ± 0.2

*Male Sprague-Dawley rats, 230–260 g, were injected ip with cyclophosphamide dissolved in a 0.9% NaCl solution; the volume injected was 3 ml/kg and doses were 100 and 50 mg/kg when concentrations of cyclophosphamide and 4-hydroxycyclophosphamide, respectively, were determined. Animals were also given 10 mg/kg of acetazolamide orally 15 mins before and 105 mins after cyclophosphamide, 660 mg/kg of NH_4Cl orally 120 mins before cyclophosphamide, or 88 mg/kg of furosemide orally 15 mins before cyclophosphamide. All diuretics were given as aqueous solutions; the volume given each time was 8.8 ml/kg. Control animals received identical volumes of water; values obtained for these animals were pooled because they were essentially identical. Half-lives were determined as described in fig 3 and Materials and Methods section. Urine was collected during the 24-hr period following cyclophosphamide injection; cyclophosphamide content of these collections was measured. Of the total cyclophosphamide excreted in the urine, > 90% was excreted in the first 12 hrs following cyclophosphamide administration. Values are the mean ± SE of measurements made on 3–10 animals.

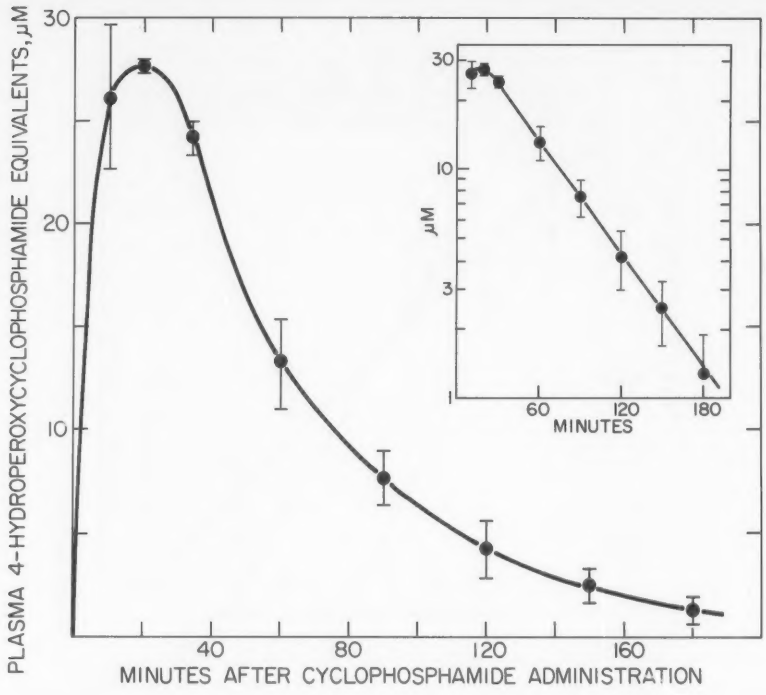


FIGURE 3.—Plasma concentrations of 4-hydroxycyclophosphamide following the administration of cyclophosphamide to rats. Male Sprague-Dawley rats, 230–260 g, were injected ip with 50 mg/kg of cyclophosphamide, and plasma concentrations of 4-hydroxycyclophosphamide were determined as described in Materials and Methods section. Values are the mean ± SE of measurements made on 5 animals. A semilog plot of the data is presented in the inset; the apparent half-life was 37 mins. The 30–150-min values, inclusive, were used to generate the apparent plasma half-lives presented in table 2 for 4-hydroxycyclophosphamide.

droxycyclophosphamide because the former readily gives rise to the latter in aqueous phosphate solution (5,13) and because 4-hydroxycyclophosphamide is relatively much less stable, making it more difficult to work with. Conversion of 4-hydroperoxycyclophosphamide to 4-hydroxycyclophosphamide does not appear to require the presence of a general base catalyst (5). Bladder weights of 178 mg were obtained when 3.8 μmols of 4-hydroperoxycyclophosphamide (19 mM) was infused, the pH was 8, and the phosphate concentration was 40 mM. Brock et al (1,3) obtained similar results using 4-hydroxycyclophosphamide. That 4-hydroperoxycyclophosphamide was not as potent as acrolein was expected, given that 4-hydroperoxycyclophosphamide must give rise to acrolein before toxicity is effected. Conversion of 4-hydroperoxycyclophosphamide to 4-hydroxycyclophosphamide in similar media takes several minutes (5,13) and the half-life of 4-hydroxycyclophosphamide in 40 mM phosphate buffer at pH 8 is about 70 mins (5). The increase in bladder weight induced by 4-hydroperoxycyclophosphamide was, as expected, dependent on the pH and the phosphate concentration of the medium; decreasing the pH or the phosphate concentration measurably decreased the potency of 4-hydroperoxycyclophosphamide (fig 5). For example, at pH 6 and a phosphate concentration of 4 mM, 20 mM 4-hydroperoxycyclophosphamide caused little or no increase in bladder weight above control; the half-life of 4-hydroxycyclophosphamide in 4 mM phosphate buffer at pH 6 is about 2000 mins (5). Brock et al (3) used 4-hydroxycyclophosphamide and observed similar pH dependency. The effects of higher concentrations of 4-hydroperoxycyclophosphamide could not be determined according to the protocol used because of solubility problems.

Phosphoramide mustard, even at the relatively high concentration of 50 mM (10 μmols), was essentially without effect with regard to increasing the bladder weight, regardless of the pH or the phosphate concentration of the infusion medium. However, there was a statistically

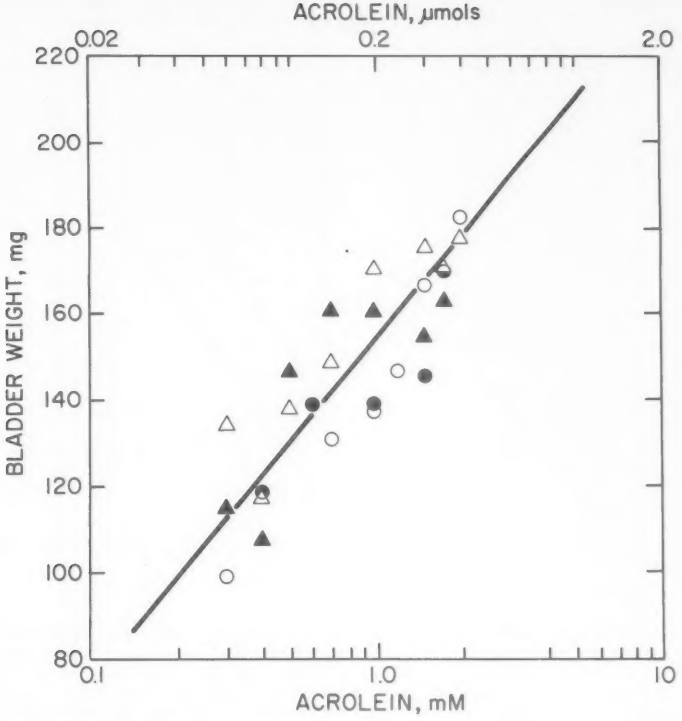


FIGURE 4.—Bladder toxicity effected by acrolein at pH 6 and 8 and at phosphate concentrations of 4 and 40 mM: dose-response relationship. Female Sprague-Dawley rats, 155–200 g, were anesthetized, their bladders were catheterized, and acrolein was infused in 0.2 ml of a 4 mM phosphate buffer solution at pH 6 (△) or 8 (▲) or a 40 mM phosphate buffer solution at pH 6 (○) or 8 (●). 1 hr later, infusates were removed, bladders were flushed with a normal saline solution, and catheters were removed. Bladder wet weights were determined 48 hrs after infusion. Acrolein was omitted from infusate to obtain control bladder weights. Values for control bladder weights are given in table 3. All values are the mean of determinations made on 2–6 animals. SE bars were omitted for purposes of clarity; they ranged from 4 to 22 mg.

significant ($P < 0.1$) increase in bladder weight when the pH was 6 and the phosphate concentration was 40 mM (table 3). Brock et al (1,3) reported similar findings.

Wagner et al (14) argue that acrolein liberated from 4-hydroxycyclophosphamide in the urine cannot be the causative agent with regard to cyclophosphamide-induced hemorrhagic cystitis, because the amounts of 4-hydroxycyclophosphamide and acrolein that they found in the urine were insufficient to account for the expected cystitis; no mention was made as to whether cystitis did indeed occur.

Urinary excretion of 4-hydroxycyclophosphamide following the administration of 50 mg/kg of cyclophosphamide ip to male rats is presented in figures 6 and 7. The assay employed is relatively specific for acrolein and compounds that give rise to acrolein, eg, 4-hydroxycyclophosphamide (7,8). We assume that the material determined to be present in the urine is largely or exclusively 4-hydroxycyclophosphamide because (*a*) acrolein generated in the plasma is likely to react immediately with plasma macromolecules and thus is not likely to be cleared as such from the plasma by the kidneys (12,15) and (*b*) the time between urine formation and collection is relatively short, so that little conversion of 4-hydroxycyclophosphamide to acrolein in the urine itself would be expected.

The urinary concentration of 4-hydroxycyclophosphamide in successive 30-min collection periods decreased exponentially and reflected the exponential decrease in plasma concentrations of 4-hydroxycyclophosphamide; the highest concentration observed was about 2 mM (fig 6). Assuming that the bladder is completely empty at the start of the experiment, that the animal does not void during the experiment, and that

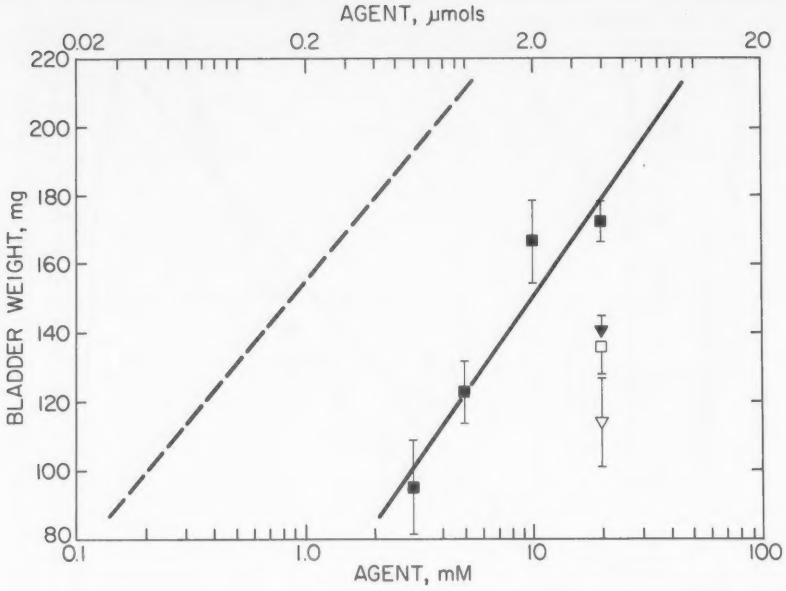


FIGURE 5.—Bladder toxicity effected by 4-hydroperoxycyclophosphamide at pH 6 and 8 and at phosphate concentrations of 4 and 40 mM. Female Sprague-Dawley rats, 155–200 g, were anesthetized, their bladders were catheterized, and 4-hydroperoxycyclophosphamide was infused in 0.2 ml of a 4 mM phosphate buffer solution at pH 6 (▽) or 8 (▼) or a 40 mM phosphate buffer solution at pH 6 (□) or 8 (■). 1 hr later, infusates were removed, bladders were flushed with a normal saline solution, and catheters were removed. Bladder wet weights were determined 48 hrs after infusion. 4-Hydroperoxycyclophosphamide was omitted from the infusate to obtain control bladder weights (values listed in table 3). All values are the mean ± SE of determinations made on 3–6 animals. The dose-response relationship between acrolein and bladder weight (broken line) is included for comparison.

4-hydroxycyclophosphamide in the urine does not decompose, the cumulative urinary concentration of 4-hydroxycyclophosphamide in the bladder is calculated to range between 1 and 2 mM during the 3-hr period following cyclophosphamide administration (fig 6, inset). About 3.5% (2 μmols) of the total cyclophosphamide dose was excreted in the urine as 4-hydroxycyclophosphamide; most of this was excreted in the 3-hr period following cyclophosphamide administration (fig 7).

The values obtained in the present experiments for urinary 4-hydroxycyclophosphamide are somewhat higher than those obtained by others (14,16–18). Probable explanations relate to the dose of cyclophospha-

TABLE 3.—Bladder toxicity of phosphoramide mustard*

pH	PO_4 (mM)	Bladder weight (mg)	
		Control	Phosphoramide mustard
6	4	119 ± 10	117 ± 8
6	40	108 ± 8	138 ± 17†
8	4	111 ± 4	115 ± 15
8	40	104 ± 11	123 ± 21

*Female Sprague-Dawley rats, 155–200 g, were anesthetized, their bladders were catheterized, and 50 mM phosphoramide mustard (10 μmols) was infused in 0.2 ml of a 4 or 40 mM phosphate buffer solution at pH 6 or 8. 1 hr later, infusates were removed, bladders were flushed with normal saline solution, and catheters were removed. Bladder wet weights were determined 48 hrs after infusion. Phosphoramide mustard was omitted from infusate to obtain control bladder weights. Bladder weight in animals not submitted to infusion was 69 ± 4 mg. All values are the mean ± SE of determinations made on 3–4 animals.

†Significantly different from control; $P < 0.1$.

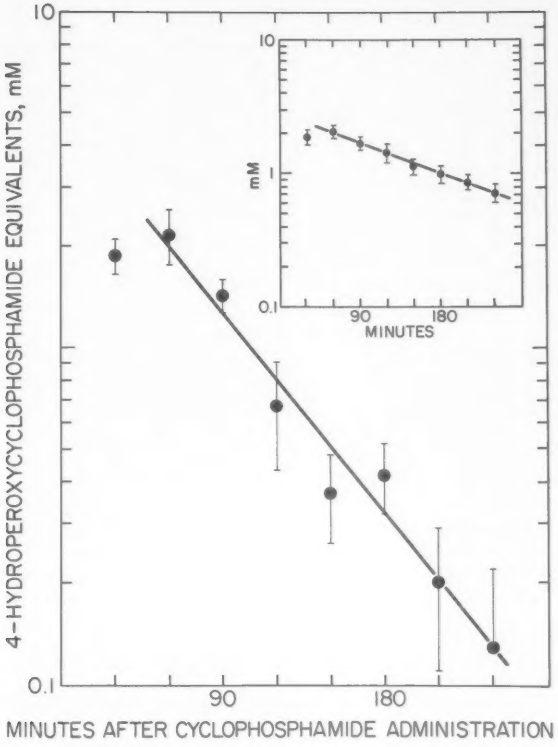


FIGURE 6.—Urinary 4-hydroxycyclophosphamide concentrations. Male Sprague-Dawley rats, 300–350 g, were prepared and injected with 50 mg/kg of cyclophosphamide as described in Materials and Methods section. Urine was collected for successive 30-min periods, and 4-hydroxycyclophosphamide concentration in each sample was determined as described in Materials and Methods section. Values are the mean ± SE of determinations made on 4 animals. Inset: Concentrations of 4-hydroxycyclophosphamide calculated to be present in bladder urine, assuming that bladder is empty at start of experiment and that animals do not void during 4-hr collection period.

mide employed, the relative rates at which 4-hydroxycyclophosphamide is generated and excreted in different species, and the stability of 4-hydroxycyclophosphamide in urine.

Administration of 50 mg/kg of cyclophosphamide ip to male and female rats increases bladder weight by about 30–40 mg/100 g of body weight (fig 1). In situ, approximately 0.26–0.42 μmol of acrolein is required to produce a similar response (fig 4). About 3.5% of the cyclophosphamide given to 325-g rats was excreted in the urine as 4-hydroxycyclophosphamide, the potential source of acrolein (fig 7). Assuming that 3.5% of the administered dose is also excreted in smaller rats, 1.0–1.7 μmols of 4-hydroxycyclophosphamide would be excreted in the urine of 150–250-g rats given 50 mg/kg of cyclophosphamide. The pH of the urine obtained from cyclophosphamide-treated male rats averaged about 6.8; the urinary inorganic phosphate and bicarbonate concentrations averaged about 32 mM and 3 mEq/L, respectively (table 1). Bicarbonate is essentially without catalytic activity at this concentration and pH (N. E. Sladek, unpublished observations), but the half-life of 4-hydroxycyclophosphamide in 32 mM phosphate at pH 6.8 is about 3 hrs (5). Thus, the amount of 4-hydroxycyclophosphamide (acrolein) excreted in the urine is clearly sufficient to account for cyclophosphamide-induced bladder toxicity.

DISCUSSION

Our investigations strongly support the view that the urotoxic effects of cyclophosphamide are caused by the acrolein liberated from 4-hydroxycyclophosphamide in the urine (1–3). The results clearly refute the contention

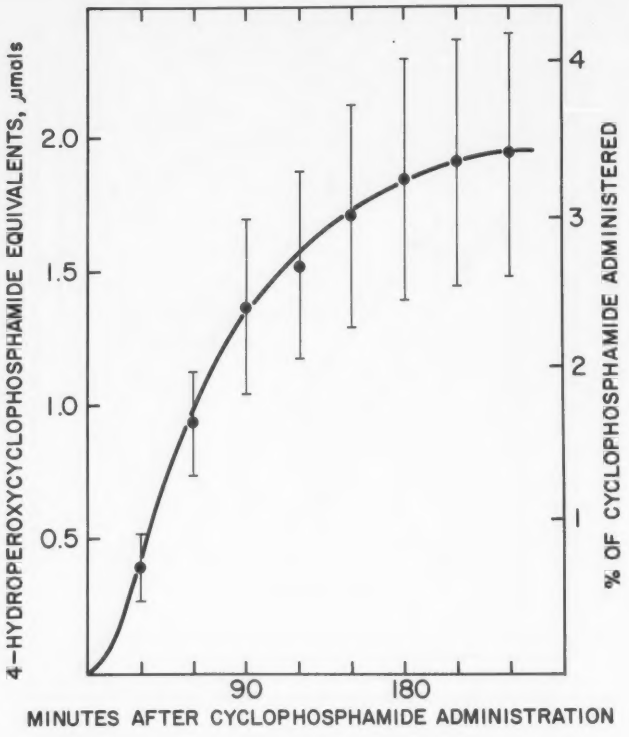


FIGURE 7.—Urinary excretion of 4-hydroxycyclophosphamide. Cumulative amount of 4-hydroxycyclophosphamide excreted in urine was determined as part of experiments presented in fig 6. Details of experiment are given in fig 6 and Materials and Methods section.

that the amounts of 4-hydroxycyclophosphamide and acrolein in the urine are insufficient to account for the toxicity (14). In all probability, only the acrolein released in the urine causes the toxic action because acrolein is very reactive and, when generated systemically, reaches the urine largely in the form of 3-hydroxypropylmercapturic acid, a relatively innocuous metabolite (12,15).

Conversion of 4-hydroxycyclophosphamide to acrolein requires the presence of a general base catalyst (4,5). Several, perhaps numerous, agents can serve in this capacity; identified general base catalysts include bicarbonate and inorganic phosphate (5). The rate at which the conversion to acrolein proceeds is highly dependent on the concentrations of these catalysts in the environment as well as on the pH of that environment; the rate increases as these environmental variables increase [(4,5); J. E. Low and N. E. Sladek, unpublished observations]. These considerations gave rise to the prediction that the urotoxic potency of 4-hydroxycyclophosphamide, and thus cyclophosphamide, would increase with increasing urinary pH and concentrations of general base catalysts (5). The experiments presented in figure 5 clearly demonstrate that this was the case.

Increased fluid intake, diuretics, alkalinization of the urine, perfusion of the bladder with nucleophiles, and systemic administration of rapidly excreted nucleophiles have all been offered as potentially useful preventive measures with regard to the urotoxic effects of cyclophosphamide (1,2,19–30). Our findings clearly indicate that alkalinizing the urine is contraindicated as is the use of certain diuretics. Specifically, whereas the use of diuretics such as NH_4Cl that do not alkalinize the urine or increase urinary general base catalyst concentration is likely to be beneficial in minimizing cyclophosphamide-induced bladder toxicity, the use of diuretics such as acetazolamide that do alkalinize the urine and/or increase urinary general base catalyst concentration may not be helpful, even though urine flow

is increased. In the case of acetazolamide, a reduction in cyclophosphamide-induced urotoxicity would be expected, due to dilution of the reactants and decreased contact time caused by the increased urine flow. This is offset, however, by the marked increase in urinary pH and bicarbonate concentration; at 100 mEq/L of bicarbonate, the half-life of 4-hydroxycyclophosphamide is about 2 hrs at pH 8 (J. E. Low and N. E. Sladek, unpublished observation), and although the inorganic phosphate concentration decreases, its catalytic activity does not change appreciably, due to the increased pH (4,5).

The reader should be aware that the dose of furosemide used in our experiments was relatively high. In man, the acute effects of a therapeutic dose of furosemide do not usually include acidification of the urine and may even include a slight increase in urine pH; increased bicarbonate excretion and phosphaturia may also be observed (31).

From the above considerations, it follows that in addition to alkalinizing agents and certain diuretics, other factors may potentially influence the urotoxic potential of cyclophosphamide by increasing urinary bicarbonate or inorganic phosphate concentrations and/or by alkalinizing the urine. Included factors are other drugs, hormonal status, disease status, diet, age, and chronorhythmic variability. For example, other agents that inhibit carbonic anhydrase would increase bicarbonate excretion and alkalinize the urine, and insulin and parathyroid hormone are known to be antiphosphaturic and phosphaturic, respectively. The renal handling of phosphate and the phosphaturic potential of agents such as calcitonin, glucagon, dopamine, and acetylcholine, as well as those of other agents, have been reviewed (32).

General base catalysts other than phosphate and bicarbonate may also be operative (5). Disease and hormonal status, diet, age, chronorhythmic variability, and drugs would also be expected to influence the concentration of these catalysts in the urine, but specific possibilities cannot be identified because the identity of the putative catalysts is not currently known.

Ifosfamide, a chemical congener of cyclophosphamide, is also urotoxic; hemorrhagic cystitis is the major dose-limiting toxic reaction to this agent. This toxicity is caused by the acrolein released in the urine from 4-hydroxyifosfamide, the primary metabolite of ifosfamide (1,3). Since this conversion also requires the presence of a general base catalyst and is identically pH-dependent (J. E. Low and N. E. Sladek, unpublished observations), the considerations made above for cyclophosphamide are likely to apply to ifosfamide as well.

Cyclophosphamide is also, apparently, a bladder carcinogen (33–37). Whether acrolein generated in the urine is the causative agent is unclear. We are unaware of any studies directed at evaluating the carcinogenic potential of acrolein. However, as pointed out by Izard and Libermann (38), acrolein can give rise to glycidal via epoxidation (39), and the carcinogenicity of glycidal has been established (40). Alternatively, acrolein may sensitize the bladder epithelium to the putative carcinogenic action of one or more of the other urinary metabolites of cyclophosphamide that are, or can give rise to, alkylating agents; increased sensitization could be the result of damage to the bladder epithelium and the subsequent rapid increase in cell division caused by acrolein. In either case, the concentration of general base catalysts in, and the pH of, the urine would be important determinants relative to the potential of cyclophosphamide and other oxazaphosphorines to induce bladder cancers.

The systemic effects of cyclophosphamide are apparently mediated by phosphoramide mustard generated from 4-hydroxycyclophosphamide within cells, at least in those cells that are substantially more sensitive to 4-hydroxycyclophosphamide than to phosphoramide mustard when these agents are added directly to the environment (41–43). A similar argument can be made for other oxazaphosphorines. Cyclophosphamide has, among antitumor and immunosuppressive agents, a relatively good therapeutic index. The basis for this desired property has not been established but apparently resides with 4-hydroxycyclophosphamide (41,42). Several possibilities have been advanced and reviewed (42,44). We have recently suggested yet another possibility (5). The other metabolite released when 4-hydroxycyclophosphamide gives rise to acrolein is phosphoramide mustard; some cells may be differentially sensitive to 4-hydroxycyclophosphamide because of a greater ability by these cells to convert 4-hydroxycyclophosphamide to phosphoramide mustard, this ability stemming from a relatively greater intracellular concentration of general base catalysts and/or pH. Our investigations in the bladder could be viewed as experiments in a model system that give credence to this possibility.

The potential importance of general base catalysts and pH in the urine and in the extracellular and intracellular spaces with regard to the localized release of toxic (carcinogenic) metabolites from relatively inert parent compounds should also be noted. These factors may account for the species, strain, organ, or cellular specificity of some agents.

REFERENCES

1. Brock N, Stekar J, Pohl J, et al. Acrolein, the causative factor of urotoxic side-effects of cyclophosphamide, ifosfamide, trofosfamide and sufosfamide. Arzneim-Forsch/Drug Res 29:659–661, 1979.
2. Cox PJ. Cyclophosphamide cystitis—identification of acrolein as the causative agent. Biochem Pharmacol 28:2045–2049, 1979.

3. Brock N, Pohl J, and Stekar J. Studies on the urotoxicity of oxazaphosphorine cytostatics and its prevention. 1. Experimental studies on the urotoxicity of alkylating compounds. Eur J Cancer 17:595–607, 1981.
4. Sladek NE, Borch RF, and Low JE. Phosphate catalyzed conversion of 4-hydroperoxycyclophosphamide and 4-hydroxycyclophosphamide to phosphoramide mustard and acrolein. Proc Am Assoc Cancer Res and ASCO 22:214, 1981.
5. Low JE, Borch RF, and Sladek NE. Conversion of 4-hydroperoxycyclophosphamide and 4-hydroxycyclophosphamide to phosphoramide mustard and acrolein mediated by bifunctional catalysts. Cancer Res 42:830–837, 1982.
6. Sladek NE, Priest J, Doeden D, et al. Plasma half-life and urinary excretion of cyclophosphamide in children. Cancer Treat Rep 64:1061–1066, 1980.
7. Alarcon RA. Fluorometric determination of acrolein and related compounds with *m*-aminophenol. Anal Chem 40:1704–1708, 1968.
8. Voelcker G, Haeglsperger R, and Hohorst H-J. Fluorometrische bestimmung von "aktiviertem" cyclophosphamid und ifosfamid im blut. J Cancer Res Clin Oncol 93:233–240, 1979.
9. Struck RF. Aldophosphamide: synthesis, characterization, and comparison with "Hohorst's aldophosphamide." Cancer Treat Rep 60:317–319, 1976.
10. Hodes ME. Carbon dioxide content (titrimetric). *In* Standard Methods of Clinical Chemistry (Reiner M, ed). New York, Academic Press, Inc, vol 1, pp 19–22, 1953.
11. Lanzetta PA, Alvarez LJ, Reinach PS, et al. An improved assay for nanomole amounts of inorganic phosphate. Anal Biochem 100:95–97, 1979.
12. Alarcon RA. Studies on the in vivo formation of acrolein: 3-hydroxypropylmercapturic acid as an index of cyclophosphamide (NSC-26271) activation. Cancer Treat Rep 60:327–335, 1976.
13. Völker G, Dräger U, Peter G, et al. Studien zum spontanzerfall von 4-hydroxycyclophosphamid und 4-hydroperoxycyclophosphamid mit hilfe der dünnschichtchromatographie. Arzneim-Forsch/Drug Res 24:1172–1176, 1974.
14. Wagner T, Heydrich D, Jork T, et al. Comparative study on human pharmacokinetics of activated ifosfamide and cyclophosphamide by a modified fluorometric test. J Cancer Res Clin Oncol 100:95–104, 1981.
15. Giles PM. The biosynthesis of 3-hydroxypropylmercapturic acid from cyclophosphamide. Xenobiotica 9:745–762, 1979.
16. Voelcker G, Wagner T, and Hohorst H-J. Identification and pharmacokinetics of cyclophosphamide (NSC-26271) metabolites in vivo. Cancer Treat Rep 60:415–422, 1976.
17. Wagner T, Peter G, Voelcker G, et al. Characterization and quantitative estimation of activated cyclophosphamide in blood and urine. Cancer Res 37:2592–2596, 1977.
18. Wagner T, Heydrich D, Voelcker G, et al. Über blutspiegel und urinausscheidung von aktiviertem cyclophosphamid und seinen deaktivierungsprodukten beim menschen. J Cancer Res Clin Oncol 96:79–92, 1980.
19. Philips FS, Sternberg SS, Cronin AP, et al. Cyclophosphamide and urinary bladder toxicity. Cancer Res 21:1577–1589, 1961.
20. Brincker H. Protection of C3H-mice against toxic effects of endoxan by means of cysteine hydrochloride. Acta Pathol Microbiol Scand 61:321–332, 1964.
21. Reynolds RD, Simerville JJ, O'Hara DD, et al. Hemorrhagic cystitis due to cyclophosphamide. J Urol 101:45–47, 1969.
22. Primack A. Amelioration of cyclophosphamide-induced cystitis. JNCI 47:223–227, 1971.
23. Botta JA, Jr, Nelson LW, and Weikel JH, Jr. Acetylcysteine in the prevention of cyclophosphamide-induced cystitis in rats. JNCI 51:1051–1058, 1973.
24. Vahlensiek W, Hoefer-Janker H, Bruhl P, et al. Zur pathogenese, klinik und prophylaxe der sogenannten cyclophosphamidzystitis. Muench Med Wochenschr 116:1889–1894, 1974.
25. Levy L, and Harris R. Effect of N-acetylcysteine on some aspects of cyclophosphamide-induced toxicity and immunosuppression. Biochem Pharmacol 26:1015–1020, 1977.
26. Cox PJ, and Abel G. Cyclophosphamide cystitis. Studies aimed at its minimization. Biochem Pharmacol 28:3499–3502, 1979.
27. Scheef W, Klein HO, Brock N, et al. Controlled clinical studies with an antidote against the urotoxicity of oxazaphosphorines: preliminary results. Cancer Treat Rep 63:501–505, 1979.
28. Löwenberg B, Abels J, and Schröder FH. Mesnum and cyclophosphamide cystitis. Lancet 2:1195, 1980.
29. Brock N, Pohl J, and Stekar J. Detoxification of urotoxic oxazaphosphorines by sulfhydryl compounds. J Cancer Res Clin Oncol 100:311–320, 1981.
30. Link H, Neef V, Niethammer D, et al. Prophylaxis of haemorrhagic cystitis due to cyclophosphamide-conditioning for bone marrow transplantation. Blut 43:329–330, 1981.
31. Puschett JB, and Goldberg M. The acute effects of furosemide on acid and electrolyte excretion in man. J Lab Clin Med 71:666–677, 1968.
32. Fleisch H, and Massry SG, eds. Renal Handling of Phosphate. New York, Plenum Medical Book Co, 1980.
33. Schmähl D, and Habs M. Carcinogenic action of low-dose cyclophosphamide given orally to Sprague-Dawley rats in a lifetime experiment. Int J Cancer 23:706–712, 1979.
34. Plotz PH, Klippel JH, Decker JL, et al. Bladder complications in patients receiving cyclophosphamide for systemic lupus erythematosus or rheumatoid arthritis. Ann Intern Med 91:221–223, 1979.
35. Fairchild WV, Spence CR, Solomon HD, et al. The incidence of bladder cancer after cyclophosphamide therapy. J Urol 122:163–164, 1979.
36. Chasko SB, Keuhnelian JG, Gutowski W, III, et al. Spindle cell cancer of bladder during cyclophosphamide therapy for Wegener's granulomatosis. Am J Surg Pathol 4:191–196, 1980.
37. Fuchs EF, Kay R, Poole R, et al. Uroepithelial carcinoma in association with cyclophosphamide ingestion. J Urol 126:544–545, 1981.
38. Izard C, and Libermann C. Acrolein. Mutat Res 47:115–138, 1978.
39. Izard C. Recherches sur les effets mutagenes de l'acroleine et de ses deux epoxydes: le glycidol et le glycidal, sur *Saccharomyces cerevisiae*. C R Acad Sci, Ser D 276:3037–3040, 1973.
40. Van Duuren BL, Orris L, and Nelson M. Carcinogenicity of epoxides, lactones and peroxy compounds. JNCI 35:707–717, 1965.
41. Brock N. Comparative pharmacologic study in vitro and in vivo with cyclophosphamide (NSC-26271), cyclophosphamide metabolites, and plain nitrogen mustard compounds. Cancer Treat Rep 60:301–307, 1976.
42. Brock N, and Hohorst H-J. The problem of specificity and selectivity of alkylating cytostatics: studies on N-2-chloroethylamidooxazaphosphorines. Z Krebsforsch Klin Onkol 88:185–215, 1977.
43. Domeyer BE, and Sladek NE. Kinetics of cyclophosphamide biotransformation in vivo. Cancer Res 40:174–180, 1980.
44. Friedman OM, Myles A, and Colvin M. Cyclophosphamide and related phosphoramide mustards. Current status and future prospects. Adv Cancer Chemother 1:143–204, 1979.

Editorial on Management of Drug Toxicity

The remarkable advances of the past 20 years in the drug treatment of malignant disease have been purchased at a price, namely the short- and long-term toxic effects of the drugs that are employed. The therapeutic index of many agents is extremely narrow and there is significant overlap between tumor cytotoxicity and toxicity for normal tissue. Emphasis in drug development has been placed upon the identification of chemicals that are actively cytocidal to cancer cells, and management of systemic toxicity by methods other than manipulation of drug dose and schedule frequently lags well behind the exploration of antitumor activity. In part, this is because modification of toxicity may require a detailed and sophisticated knowledge of drug pharmacology and a clear appreciation of whether the mechanisms of tumor cell kill and organ toxicity can be separately manipulated. Such studies are not likely to be undertaken until a drug has proved its worth in the clinic. However, as a drug or class of agents becomes well-established, interest rises in ameliorating its specific toxicity in order to improve the therapeutic index. Alkylating agents, anthracyclines, platinum analogs, and antimetabolites all have characteristic organ toxicities, and in each case research programs exist with the aim of understanding and eliminating these side effects. For the hemorrhagic cystitis produced by cyclophosphamide and its congener ifosfamide, work over the past decade has emphasized the role of sulfhydryl compounds such as *N*-acetylcysteine as well as the induction of a liberal diuresis to prevent this side effect. While studies in animals and man (1–3) indicate that systemic or intravesical instillation of sulfhydryl compounds may reduce cyclophosphamide-induced cystitis, these have never been widely used procedures, in part because systemic use of sulfhydryl compounds may also reduce the antitumor activity of the drugs (4). Another standard recommendation is to induce diuresis, either with parenteral or oral fluids, to assist the excretion of cyclophosphamide metabolites and reduce their concentration in the urine. In the preceding article, Sladek and colleagues (5) suggest that acidification of the urine is desirable as well. Their studies in rats indicate that release of urotoxic acrolein is increased in the presence of general base catalysts such as phosphate and bicarbonate and that this reaction proceeds more effectively at an alkaline pH. Diuretics that acidify the urine minimize or eliminate the urotoxicity of cyclophosphamide, while acetazolamide, which alkalinizes the urine, does not alter toxicity. Systemic action of the drug is not altered in either case. These findings deserve study in man, as they may have immediate significance regarding the use of cyclophosphamide, especially in high doses. This may be a point at which the mechanisms of toxicity and antitumor activity of cyclophosphamide and its congeners diverge and can be independently managed.

Such findings emphasize the need for continued alertness by clinical investigators to the nuances of drug pharmacology. Other drugs, notably the platinum compounds and methotrexate, are also urotoxic. Recently Ozols et al (6) noted that the renal toxicity of high cisplatin doses could be ameliorated by the concurrent administration of hypertonic saline. If this finding is confirmed it will be another useful addition to our knowledge in the use of platinum compounds. On the other hand, it is known that methotrexate crystallizes in renal tubules at acid pH and that alkalinization of the urine is an important part of the prevention of renal damage by high-dose methotrexate (7).

Other investigators are currently examining the mechanisms and means of eliminating important drug toxicity such as the cardiac toxicity of anthracyclines, the severe cutaneous damage of anthracyclines and actinomycin, and the myelotoxicity of antimetabolites and alkylating agents. Since the recognition of novel new drugs for treatment of cancer is often a slow process, some of the important gains in the near future may come from learning to diminish toxicity and thereby widen the therapeutic index of the anticancer drugs presently available.

REFERENCES

1. Primack A. Amelioration of cyclophosphamide-induced cystitis. JNCI 47:223–227, 1971.
2. Botta JA, Nelson LW, and Weikel JH. Acetylcysteine in the prevention of cyclophosphamide-induced cystitis in rats. JNCI 51:1051–1058, 1973.
3. Scheef W, Klein HO, Brock N, et al. Controlled clinical studies with an antidote against the urotoxicity of oxazaphosphorines: preliminary results. Cancer Treat Rep 63:501–505, 1979.
4. Brock N, Pohl J, and Stekar J. Detoxification of urotoxic oxazaphosphorines by sulfhydryl compounds. J Cancer Res Clin Oncol 100:311–320, 1981.
5. Sladek NE, Smith PC, Bratt PM, et al. Influence of diuretics on urinary general base catalytic activity and cyclophosphamide-induced bladder toxicity. Cancer Treat Rep 66:1889–1900, 1982.
6. Ozols RF, Javadpour N, Messerschmidt GL, et al. Poor prognosis non-seminomatous testicular cancer: an effective "high-dose" cis-platinum regimen without increased renal toxicity. Proc ASCO 1:113, 1982.
7. Pitman SW, Parker LM, Tattersall MHN, et al. Clinical trial of high-dose methotrexate (NSC-740) with citrovorum factor (NSC-3590)—toxicologic and therapeutic observations. Cancer Chemother Rep Part 3, vol 6(1):43–49, 1975.

Paul V. Woolley

Georgetown University Medical Center
Vincent T. Lombardi Cancer Research Center
Washington, DC 20007

September 14, 1982

Intracavitary Bleomycin in the Management of Malignant Effusions: A Multicenter Study[1]

M. J. Ostrowski[2,*] and G. M. Halsall[3]

The efficacy of intracavitary bleomycin in preventing the recurrence of malignant effusions following aspiration was assessed in a multicenter study. Of 200 patients treated, 158 were evaluated for response at 30 days. The overall response rate was 58%, with pleural effusions responding better (62%) than peritoneal effusions (47%). Pleural effusions resulting from primary breast tumors showed the best response (72%). No side effects were seen in 79.5% of the 200 patients. Pain and transient fever were reported in 21% of the patients after intraperitoneal instillation but in only 5% of those receiving intrapleural instillations. Nausea was experienced by 5.5% of the patients. There was no evidence of myelosuppression in any patient nor of enhancement of myelosuppression in the 57 patients receiving concurrent cytotoxic therapy. There was one possible treatment-related death in an elderly man given 120 mg of bleomycin intrapleurally. This leads us to recommend that the maximum dose should be 60 mg, especially as the response rate was not improved by doses > 60 mg. We conclude that bleomycin should be the agent of choice when the instillation of a cytotoxic agent following the drainage of a malignant effusion is indicated, since it is effective in preventing recurrence of the effusion, is generally free from systemic effects, and can be given to myelosuppressed patients or those already undergoing systemic cytotoxic therapy. [Cancer Treat Rep 66:1903–1907, 1982]

Serous effusions result when the dynamic balance between fluid formation and resorption within the serous space is disrupted. The frequency with which effusions occur as a complication of progressive neoplastic disease has not been determined, but Leff et al (1) stated that 25% of all effusions are due to neoplasia. Relief of dyspnea, cough, and pain usually follows aspiration of the effusion, but reaccumulation of fluid is rapid. In one study (2) of 94 patients treated by thoracentesis alone, the average time for reaccumulation of the effusion was 4.2 days, with a 97% chance of recurrence within 1 month. Repeated aspirations may induce hypoproteinemia, which can accelerate the reaccumulation of fluid.

Different techniques have been used to prevent or delay the recurrence of malignant serous effusions, including closed-tube thoracostomy with supplementary suction, external radiotherapy, or instillation of one of a variety of agents such as radioactive isotopes and sclerosing and cytotoxic drugs. Response rates of 50%–70% have been obtained using these forms of treatment (2), but there are problems associated with their use: tube thoracostomy is a time-consuming technique; radioactive isotopes subject both hospital personnel and fellow patients to the risk of radioactive exposure; cytotoxic agents may give rise to bone marrow suppression, nausea, and vomiting; and sclerosing agents tend to be poorly tolerated because of pain.

Because of these disadvantages there is a need for an agent that is effective, easy to administer, and free from side effects. Bleomycin is a cytotoxic agent that has been shown to be effective in a number of small studies (3,4–8), and so a multicenter study was begun to investigate further the role of intracavitary bleomycin in a larger number of patients with malignant serous effusions secondary to a variety of tumors and to determine the optimum dose for instillation.

PATIENTS AND METHODS

Two hundred patients with histologically documented malignancy and pleural and/or peritoneal effusions were entered into this study from 44 centers in the United

[1]Received July 31, 1981; revised June 24, 1982; accepted Aug 5, 1982.
[2]Norfolk and Norwich Hospital, Norwich, England.
[3]Lundbeck Limited, Luton, Bedfordshire, England.

Reprint requests to: Dr. M. J. Ostrowski, Department of Radiotherapy and Oncology, Norfolk and Norwich Hospital, Brunswick Rd, Norwich, Norfolk, NR1 3SR, England.

Kingdom, each clinician treating between three and five patients. The aim of the study was to evaluate the use of simple pleural aspiration and peritoneal drainage followed by intracavitary bleomycin in busy general hospitals, as well as in the more academic units. The information recorded on entry to the study included age and sex of the patient, duration of the effusion, site and histology of the primary tumor, cytologic examination of the effusion, details of concurrent treatment, and status of the patient's primary tumor.

Aspiration Techniques

Pleural effusions.—Drainage was as complete as possible, as confirmed by chest X-ray, with appropriate precautions taken when the volume exceeded $1^1/_2$ L. Drainage was followed by the instillation of bleomycin in 100 ml of physiologic saline. The most common technique used was cannula or needle aspiration over 30–60 minutes.

Peritoneal effusions.—Gravity drainage to dryness over 12–24 hours was followed by the instillation of bleomycin in 100 ml of physiologic saline.

Dose.—Inadequate data were available regarding the most effective dose to use. Clinicians were asked to select, at their own discretion, a dose within the range of 30–180 mg.

There is no standard assessment of response recognized in the literature; in this survey the criteria used by Paladine et al (4) were adopted so that our results would be comparable. A complete response was defined as no further reaccumulation of fluid within 30 days, and a partial response was defined as asymptomatic, minimal fluid recurrence not requiring a repeat aspiration within 30 days. Failure was defined as reaccumulation of the fluid within 30 days, requiring further aspiration. Recurrences of effusion were determined by regular clinical assessment and chest X-rays. A full assessment of response to bleomycin was made at 1 month (30 days) but long-term results were not routinely recorded in view of the variety of primary tumor types treated, the different stages of the disease on entry, and the limited life expectancy of many patients. However, some clinicians were able to provide follow-up information at 60 and 90 days after instillation of bleomycin.

Of the 200 patients with malignant effusions entered into this study, 42 were excluded from analysis for the following reasons: ten died of progressive disease < 30 days after treatment with bleomycin and before response could be assessed, 26 died of progressive disease in < 30 days but without recurrence of their effusion, and six, all without recurrence, were reviewed prematurely before the 30-day assessment period (range, 17–29 days). This left 158 evaluable patients, seven of whom were lost to follow-up and have been included as nonresponders for the purpose of this analysis. Of these 158 patients, there were 66 with primary breast tumors, 30 with ovarian lesions, 27 with lung cancer, and 35 with miscellaneous tumors.

RESULTS

The overall response rate at 30 days was 58% (91 patients), 35% complete responders and 23% partial. Pleural effusions responded better (62%) than peritoneal effusions (47%) (table 1). This may be because in advanced disease, peritoneal deposits invariably become large and confluent so that the opportunity for uniform contact between drug and tumor may be less favorable in the peritoneal cavity than in the pleural space. Another reason might be obstruction of hepatic lymphatics, which would not be expected to respond to intraperitoneal therapy.

Pleural effusions associated with breast primaries were the most responsive to intracavitary bleomycin, with a response rate of 72%. Only 37% of the patients with pleural effusions secondary to lung tumors responded,

TABLE 1.—Response rate by site of primary tumor and serous cavity affected (n = 158)

Site of effusion	Site of primary tumor: Breast CR	Breast PR	Breast CR + PR	Ovary CR	Ovary PR	Ovary CR + PR	Lung CR	Lung PR	Lung CR + PR	Miscellaneous CR	Miscellaneous PR	Miscellaneous CR + PR	Response rate by site of effusion CR	PR	CR + PR
Pleural	46%	26%	72% (n = 54)	—	2 of 3	2 of 3	22%	15%	37% (n = 27)	36%	32%	68% (n = 22)	37%	25%	62% (n = 106)
Peritoneal	45.5%	18.5%	64% (n = 11)	22%	17%	39% (n = 23)		—		31%	15%	46% (n = 13)	30%	17%	47% (n = 47)
Pleural and peritoneal	1 of 1		1 of 1	1 of 4	1 of 4	2 of 4		—			—		2 of 5	1 of 5	3 of 5
Response rate by site of primary tumor	47%	24%	71% (n = 66)	20%	23%	43% (n = 30)	22%	15%	37% (n = 27)	34%	26%	60% (n = 35)			

but this included eight of ten patients with squamous cell tumors, which, in view of the well-known affinity of bleomycin for squamous cells, may indicate a specific local effect. Of the pleural effusions due to miscellaneous tumors, 68% responded although the sensitivity of individual tumor types cannot be determined from such a small series (table 2).

There were 47 patients with ascites available for evaluation, of whom 22 (47%) responded to treatment with bleomycin. As in the case of pleural effusions, patients with primary carcinoma of the breast responded well, with a response rate of 64% (seven of 11). Five patients (one with a breast primary and four with ovarian primaries) had both pleural and peritoneal effusions. Three of these patients responded to treatment, with relief of both pleural and peritoneal effusions.

The duration of response cannot be fully assessed in this preliminary evaluation. Of the 91 responders at 30 days, 66 patients were symptom-free at 60 days, five had died, six had been retapped, and 14 were lost to follow-up. Of the 66 symptom-free patients, on whom complete follow-up information was available at 60 days, 32 were still alive and well with no further effusion at 90 days, seven had died, six had been retapped, and 21 were lost to follow-up.

Toxicity

No side effects were reported in 159 (79.5%) of the 200 patients entered into this study. Pain and fever were the most commonly reported side effects, occurring in 10.5% of the patients. In most cases, these two symptoms occurred together. A total of 21% of the patients receiving intraperitoneal bleomycin experienced pain, in contrast to only 5% of those given the drug intrapleurally. Pain was controlled by mild analgesics in seven cases but was severe in another seven. (The severity of pain in the remaining seven cases was not recorded.) The causes of pain may have included irritation by bleomycin, producing an inflammatory response; the cannulation procedure; or injection of bleomycin into the abdominal wall. Gastrointestinal side effects were experienced in 5.5% of the patients (diarrhea in one, vomiting in two, and nausea in eight), whereas erythema and dyspnea, which have occasionally been seen with the parenteral administration of bleomycin, were each reported in only one patient. Two of the three patients who developed alopecia were receiving concurrent chemotherapy with cyclophosphamide. There was one death, possibly related to treatment, in a 75-year-old man given 120 mg of bleomycin intrapleurally. He became ill on the following day, with labored respiration, a rapid pulse, and a blood pressure of 116/60. The ECG was suggestive of a myocardial infarction, and he died 3 days later.

Dose

The dose of bleomycin varied between 30 and 180 mg, with 60, 90, and 120 mg being most frequently used. Of 81 patients receiving a dose of 60 mg, 52 showed a response and 29 did not. Fifteen of 29 patients who were given a dose of 90 mg responded, and 120 mg produced a response in 11 of 19 patients. There was no difference in the mean dose of bleomycin given to responders (mean, 72 mg; range, 30–180) and nonresponders (mean, 74 mg; range, 30–150). The median dose was 60 mg in both responders and nonresponders, which suggests that 60 mg is a reasonable routine dose to administer.

TABLE 2.—Sites of miscellaneous primary tumors and response of effusion to bleomycin

	Pleural effusions (n = 22)		Peritoneal effusions (n = 13)	
	Responders	Nonresponders	Responders	Nonresponders
Colorectal adenocarcinoma	2	—	1	2
Esophageal adenocarcinoma	—	—	—	1
Gastric adenocarcinoma	—	—	—	1*
Non-Hodgkin's lymphoma	3	—	—	—
Hodgkin's disease	1	—	—	—
Melanoma	1	—	—	—
Adamantinoma	1	—	—	—
Osteosarcoma	—	1	—	—
Mesothelioma	3	3	—	—
Endometrial carcinoma	1	1	3	—
Unknown primary	3	2*	2	3

*1 patient lost to follow-up and included as a nonresponder.

The development of a malignant effusion indicates progression of disease, even though in some cases the patient is in good general health and may have many months of good-quality life remaining. In other patients, however, it signifies the entry into the terminal phase of their illness, so their life expectancy is limited. In this series of patients, 76 had uncontrolled advancing disease (ie, the development of an effusion was only one of several signs of progressive disease), in 57 the other signs of disease were present but static, and in 17 the effusion was the only sign of progressive disease. Table 3 shows the response of the effusion to intracavitary bleomycin in relation to the other signs of the patient's disease. The patients in whom an effusion was the only sign of advancing disease responded well to bleomycin therapy (16 of 17 patients). The effusion was controlled in 39 of the 76 patients with uncontrolled progressive disease and in 36 of the 57 patients in whom the other signs of disease were static. Whether or not other signs of disease are progressing or in remission appears therefore to be a prognostic factor of importance for prediction of response to bleomycin ($P < 0.01$).

Many patients were receiving concurrent systemic chemotherapy for their disease, and it could be argued that this treatment may have influenced the effusion-free interval. However, systemic chemotherapy was not changed prior to or during the trial, so that in some patients the effusion was accumulating despite concurrent chemotherapy. In fact, there were only small differences between the concurrent chemotherapy given to those patients whose effusion was controlled by bleomycin (bleomycin responders) and those patients whose effusion was not controlled by bleomycin (bleomycin nonresponders). For instance, 13% of bleomycin responders were given corticosteroid, 9% were given diuretics, and 1% received estrogens. Among bleomycin nonresponders, 18% were given corticosteroid, 17% received diuretics, and 5% had radiotherapy.

Concurrent cytotoxic chemotherapy was administered to 57 patients in whom there was no evidence of enhanced myelosuppression. The percentages of patients who received cytotoxic chemotherapy and whose effusion was controlled or not by intracavitary bleomycin are similar (37% and 38%, respectively). Twenty-one patients, all with carcinoma of the breast, were taking the antiestrogen tamoxifen, and the control of effusion by bleomycin in this group was very good (81%). This response rate is comparable to that seen in the whole group of patients with breast cancer (71%). Of the bleomycin responders, 37% were actually receiving no concurrent chemotherapy, compared with only 28% of the bleomycin nonresponders. These figures indicate that the control of effusion in this series of patients was due not to systemic chemotherapy but to intracavitary bleomycin.

The mean and median duration of effusion before treatment with bleomycin was the same in responders (mean, 4 months; median, 1; range, 0.5–36) and nonresponders (mean, 3.2 months; median, 2; range, 0.5–25). Age was not a determining factor in predicting response, the mean age being 60 years (range, 33–82) in responders and 61 years (range, 17–84) in nonresponders.

Cytologic assessment of the effusion was not done in 35% of the patients (33% of the responders and 37% of the nonresponders) and may not, therefore, be representative of the whole group of patients. However, of the responders, 77% had malignant cells in the aspirated fluid and 23% did not. Of the nonresponders, 74% were cytologically positive and 26% negative. Bleomycin appears therefore to be equally effective both in the presence and absence of malignant cells in the effusion.

TABLE 3.—Status of other signs of malignancy*

Status of other signs of malignancy	Effusion responding to intracavitary bleomycin ($n = 91$)	Effusion not responding to intracavitary bleomycin ($n = 60$)†
Progression	39 (43%)	37 (62%)
No change	36 (39.5%)	21 (35%)
Remission	16 (17.5%)	1 (1.5%)

*$\chi^2 = 10.927$; df = 2; $P < 0.01$.
†Information not available for 1 patient.

DISCUSSION

Cunningham et al (3) first reported the use of intracavitary bleomycin in the management of malignant effusions in 12 patients, of whom five (48%) showed a complete response. Patient tolerance was described as excellent; only one person reported a transient mild erythematous rash, and there was no exacerbation of leukopenia, which was present in two patients prior to treatment. Paladine et al (4) confirmed this initial impression in their series of 51 patients who had an overall response rate of 59% at 30 days. Pleural effusions responded better than peritoneal effusions, with response rates of 63% and 36%, respectively; 70% of the pleural effusions secondary to primary breast carcinoma responded. Toxicity was described as minimal. Alopecia, skin changes, and pulmonary fibrosis were not seen despite evidence of systemic absorption; pain, nausea, rashes, or hypotension occurred in only 2% of the patients; and ten patients (20%) experienced a transient fever. Toxicity was more common following intraperitoneal instillation and was thought to be due to the greater drug absorption from the peritoneal cavity (5,9). The absence of hematologic toxicity and local discomfort led the authors to conclude that bleomycin is an effective alternative to quinacrine for the control of pleural effusions in the leukopenic patient.

Three abstracts also reported the use of intracavitary bleomycin, all in small series of patients. Lippman et al (6) claimed a 92% response rate (11 of 12 patients) in pleural effusions but three of three peritoneal effusions failed to respond. There were no toxic reactions. Trotter et al (7) reported to the British Association of Cancer Research a study of 22 patients who received intracavitary bleomycin for control of malignant effusions: 78% of pleural effusions and 69% of peritoneal effusions responded. There were two possible drug-related deaths, one of which was associated with an unusually high serum level of bleomycin. This led them to recommend that the dose of bleomycin not exceed 40 mg/m^2 in the elderly. In our series there was also one possible drug-related death in a patient given 120 mg of bleomycin intrapleurally, and we endorse this recommendation, particularly as the response rate is not improved by doses > 60 mg. A study by Gupta et al (8) comparing intrapleural bleomycin in 13 patients and intrapleural tetracycline in 12 patients showed response rates of 54% (seven of 13) and 58% (seven of 12), respectively. They concluded that the agents are equally effective with minimal toxicity.

The mechanism of action of intracavitary bleomycin is unknown. Systemic absorption of intracavitary bleomycin is approximately 45% of the instilled dose (9). The half-life after intraperitoneal instillation is significantly longer than after intrapleural instillation, which in turn is significantly longer than after iv injection (9). The longer half-life following intraperitoneal instillation as compared with intrapleural instillation may be due to incomplete drainage of peritoneal fluid prior to instillation of bleomycin. Dedrick et al (10) suggest that the systemic absorption of a drug from the peritoneal cavity is dependent on the volume of peritoneal fluid: the larger the residual peritoneal fluid volume, the lower the rate of drug absorption.

Bleomycin may therefore remain in contact with the abdominal serous membrane for a relatively long time, and this might explain the more frequent observation of pain after intraperitoneal instillation (21%) than after intrapleural instillation (5%) in this study. However, pleural effusions respond better than peritoneal effusions to intracavitary bleomycin, so this extended contact of the serous membranes with the drug does not appear to be necessary for bleomycin to exert its therapeutic effect. The fibrous adhesions found at autopsy suggest that bleomycin may stimulate an irritative pleuritis, although a local cytotoxic effect cannot be ruled out (4). An interesting observation in our study was that eight of ten patients with pleural effusions secondary to squamous cell lung primaries responded, suggesting that bleomycin may have a local cytotoxic effect.

The place of bleomycin in the management of malignant effusions can be determined only by controlled comparative clinical trials, using standard aspirating techniques and carefully defined response criteria. However, the results of this study in a large number of patients confirm that bleomycin is effective in the palliation of serous effusions arising secondary to a wide range of malignancies, and indicate that it may have advantages over other agents. The drug is well-tolerated, which is an important factor in treating patients with advanced malignant disease in whom myelosuppression, pain, nausea, and vomiting would detract from their quality of life. For these reasons, we conclude that bleomycin should be the agent of choice when the instillation of a cytotoxic agent following the aspiration of a malignant effusion is considered appropriate.

REFERENCES

1. Leff A, Hopewell PC, and Costello J. Pleural effusions from malignancy. Ann Intern Med 88:532–537, 1978.
2. Anderson CB, Philpott GW, and Ferguson TB. The treatment of malignant pleural effusions. Cancer 33:916–922, 1974.
3. Cunningham TJ, Olson KB, Horton J, et al. A clinical trial of intravenous and intracavitary bleomycin. Cancer 29:1413–1419, 1972.
4. Paladine W, Cunningham TJ, Sponzo R, et al. Intracavitary bleomycin in the management of malignant effusions. Cancer 38:1903–1908, 1976.
5. Alberts D, Chen HS, Liu R, et al. Systemic absorption of bleomycin after intracavitary administration. Proc Am Assoc Cancer Res and ASCO 19:305, 1978.
6. Lippman AJ, Cohen FB, and Custodio MC. A clinical trial of intracavitary bleomycin. Proc Am Assoc Cancer Res and ASCO 16:1104, 1975.
7. Trotter JM, Stuart JFB, McBeth F, et al. The management of malignant effusions with bleomycin. Br J Cancer 40:310, 1979.
8. Gupta N, Oppell RW, Padova, J et al. Intrapleural bleomycin versus tetracycline for control of malignant pleural effusions: a randomised study. Proc Am Assoc Cancer Res and ASCO 21:189, 1980.
9. Alberts DS, Chen HSG, Mayersohn M, et al. Bleomycin pharmacokinetics in man. II. Intracavitary administration. Cancer Chemother Pharmacol 2:127–132, 1979.
10. Dedrick RL, Myers CE, Bungay PM, et al. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat Rep 62:1–11, 1978.

Hydroxy-9-methyl-2-ellipticinium for Osseous Metastases From Breast Cancer: A 5-Year Experience[1]

P. Juret,* J. F. Heron, J. E. Couette, T. Delozier, and J. Y. Le Talaer[2]

A group of 135 patients with osseous metastases from breast cancer were treated with hydroxy-9-methyl-2-ellipticinium (100 mg/m^2 weekly). Although it was impossible to grade the response precisely, because only indirect criteria are available for assessing the course of bone metastases (radiographs, quantified ^{99m}Tc pyrophosphate scintigrams, CEA), it was considered that an objective response was obtained in 44 cases. These responses lasted from 3 to 17 months. The main characteristic of the compound is its lack of marrow toxicity, a valuable property in osseous lesions, where frequent marrow involvement makes it difficult to use conventional drugs. The major and most unpleasant side effect was an inhibition of salivary secretion, which causes other complications such as tongue mycosis, anorexia, and asthenia. Immunologic disorders were less frequent, and four patients developed severe tubular renal insufficiency. [Cancer Treat Rep 66:1909–1916, 1982]

The antitumor action of ellipticine and its derivatives in animals has been reported by several authors (1–4). In human oncology, methoxy-ellipticine has been used successfully in the treatment of acute myeloblastic leukemia (5,6). In previous papers, our group pointed out the therapeutic value of hydroxy-9-methyl-2-ellipticinium (hereafter referred to as elliptinium) in advanced cancers, especially in breast cancers (7–10) (fig 1). This compound was synthesized and studied in experimental tumors by the Laboratoire de Chimiothérapie Expérimentale, Institut Gustave Roussy (Villejuif, France) and the Institut de Chimie des Substances Naturelles, Centre National de Recherches Scientifiques (Gif-sur-Yvette, France) (2–4). It is currently being used in phase II and III trials in several countries. The phase II studies have demonstrated some activity of elliptinium in thyroid and kidney carcinomas, in soft tissue sarcomas, and mainly in advanced breast cancers (8,9,11,12). However, to our knowledge, these trials have not included an evaluation of the effect of the compound on osseous metastases. This exclusion is understandable, since it is difficult to assess and measure the effect of any treatment on metastatic bone lesions because investigators have recourse only to indirect criteria (X-ray films, ^{99m}Tc pyrophosphate scintigrams, and biologic markers such as CEA).

These methods preclude grading responses by the criteria typically used in phase II trials, ie, complete remission, partial remission (> 50% and < 50%), or no remission. Nonetheless, osseous metastases are the most frequent secondary lesions of breast cancer, and we previously drew attention to the favorable effect of el-

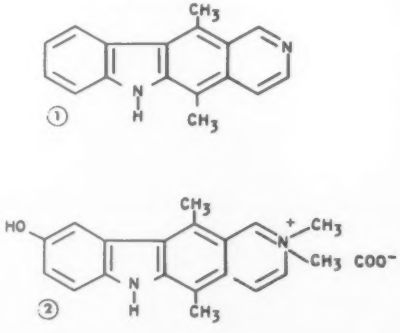


FIGURE 1.—Chemical structure of ellipticines: 1. ellipticine; and 2. hydroxy-9-methyl-2-ellipticinium acetate (elliptinium).

[1]Received Oct 2, 1981; revised Aug 3, 1982; accepted Aug 19, 1982.
[2]Departments of Medicine (*P. Juret* and *J. F. Heron*), Nuclear Medicine (*J. E. Couette*), and Radiation (*T. Delozier*), and Biochemistry Laboratory (*J. Y. Le Talaer*), Centre François Baclesse, Caen, France.
**Reprint requests to:* Dr. Paul Juret, Medicine Department, Centre François Baclesse, Route de Lion-sur-Mer, F-14021 Caen Cedex, France.

liptinium in this sort of malignancy (7,8). This therapeutic value of elliptinium was enhanced by its low hematologic toxicity, a useful property in bone metastases, where the frequent involvement of the marrow often limits the use of conventional drugs. These considerations encouraged us to pursue the study of the effect of elliptinium in osseous metastases from breast cancer, in spite of the difficulty in assessing response.

MATERIALS AND METHODS

Between June 1976 and June 1981, 328 patients with advanced cancers were treated with elliptinium at the Centre François Baclesse (CFB). Among these patients, 183 had advanced breast carcinomas; in 137 of these, skeletal involvement was apparently the sole metastatic process as demonstrated by clinical examination, hepatic isotopic and/or ultrasonic scans, liver enzymes, and lung roentgenograms. Except for the first eight patients, who were initially included in phase I trial and received elliptinium at weekly doses of 4–80 mg/m^2, all patients were given a 60–90-minute weekly infusion of 100 mg/m^2 of elliptinium in an isotonic glucose solution. We chose the dose of 100 mg/m^2/week because our phase I study showed that this dose was usually well-tolerated (8). Two of the 137 patients were excluded from our study: one erroneously received an associated systemic treatment; the other was the only male in this series and his response will be considered separately. Therefore, a total of 135 women were included in this study. For eight patients, elliptinium had to be discontinued before the sixth week of treatment, because of intolerance (seven patients had digestive disorders, and one had hemolysis resulting from a rise of anti-elliptinium antibodies); they were considered failures. The remaining 127 women received a minimum of six weekly infusions, the treatment being continued until it became obvious that elliptinium was ineffective or that its effectiveness had ceased after a time of response. Treatment ranged from 6 to 86 weekly infusions.

With the exception of women known to have no estrogen receptors in their breast tumors, all patients underwent an endocrine treatment as a first systemic procedure (castration, tamoxifen). Chemotherapy was administered only after failure to respond to the endocrine treatment was ascertained or in the case of recurrent disease. From June 1976 to October 1978, patients were given elliptinium only after they had failed to respond to conventional chemotherapy (doxorubicin, cyclophosphamide, antimetabolites, etc) or when these cytostatic agents had to be discontinued due to their toxic effects (series 1). From October 1978, when it became clear that elliptinium could induce remission without marrow toxicity, the order of these last procedures was reversed: elliptinium became the first-line cytostatic treatment and conventional chemotherapy the second-line treatment (series 2). We excluded from this trial only patients whose poor vein system made cyclic infusions difficult. A total of 69 patients were treated in series 1 and 66 in series 2. No radiotherapy or other anticancer treatment was given jointly with elliptinium.

The main difficulty of this study was in the assessment of an objective remission, as the improvement or aggravation of osseous metastases can generally be established only by indirect methods. We based our evaluation on three different investigations:

(*a*) Monthly roentgenograms of the skull, whole spine, and pelvis (and other sites when necessary).

(*b*) Monthly ^{99m}Tc pyrophosphate scintigrams. The results were quantified according to the method elaborated at the CFB: the ratio of the uptake of this marker on healthy bones to the uptake on knees ranges normally from 1 to 2.5 but is usually higher in the case of metastatic lesion; aggravation or improvement results respectively in a rise or fall of the initial ratio (13).

(*c*) Twice monthly, CEA level was assayed (normal value: < 20 ng/ml).

The difficulty in evaluating an objective remission was increased by the fact that the above criteria were not constantly found. Of the 135 patients, 31 had normal scintigrams and 54 had normal CEA levels. Radiographic evidence of disease was present *in all patients*, but the X-ray images were difficult to compare in 25 cases, mainly when the lesions were situated in the sacropelvic area.

Given these conditions, response to elliptinium was rated as positive when the following occurred.

(*a*) Improvement according to *all the available criteria* and demonstrated by the above methods had lasted > 3 months: ie, an improvement of osteolytic or osteoblastic images on X-ray films *whatever the extent of that improvement*, a drop in an abnormal ^{99m}Tc pyrophosphate uptake ratio to the normal level, and a drop in CEA to < 20 ng/ml. In patients for whom two or three tests were available, a discrepancy of the results (eg, an improvement of CEA levels and scintigrams, but an aggravation of X-ray images) disqualified them from being considered as having positive responses. The three criteria were available for 58 patients, whereas only two criteria were available for 62 patients and only one criterion was available for 15 patients (table 1).

(*b*) No new metastatic lesion had appeared in any site (osseous, visceral, soft tissue, etc) during this period.

We are fully aware that this method of evaluation is subject to criticism, but the criticism is more theoretic than practical. In no case did we have any difficulty in classifying response as a remission or a failure.

RESULTS

Therapeutic Results

Therapeutic results are summarized in tables 1 and 2. Some examples of radiographic, scintigraphic, or biologic (CEA) remissions are given in figures 2–7.

In all cases, a partial or complete subjective remission was obtained with the objective response, with disappearance of pain and return to normal or subnormal activity. Response to treatment was demonstrated radiographically in 37 of 116 cases with evaluable images: in 34 of 103 cases with osteolytic images and in three of 13 cases with osteoblastic images. The other seven objective responses (images not evaluable on X-ray films) were demonstrated by the normalization of bone scans (two cases), CEA level (one), or both (four) (table 1). A relapse developing after a response was never ascertained on X-ray images but rather by a rise of CEA level, by an aggravation of scintigram, or by visceral or soft tissue metastases. No relationship could be found between response to elliptinium and previous response to hormonal treatment. The only male treated with elliptinium was excluded from the above series. He had an objective response which lasted 13 months.

As mentioned above, the present method of evaluation based on indirect criteria did not permit us to grade the responses. The only parameter that appeared to be

TABLE 1.—Objective responses to elliptinium in osseous metastases from breast cancer, in relation to the tests available for evaluation

Evaluation criteria	No. of patients		
	Total	Responding	With disease progression
1 test	15	4	11
X-ray films	6	1	
Scintigrams	5	2	
CEA	4	1	
2 tests	62	20	42
X-ray films + scintigram	37	9	
X-ray films + CEA	15	7	
Scintigram + CEA	10	4	
All 3 tests	58	20	38
X-ray films + scintigram + CEA			

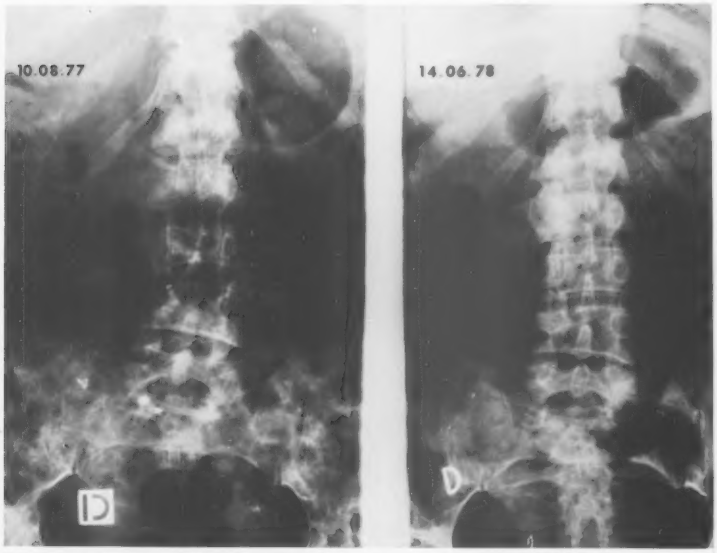


FIGURE 2.—Objective response after 10 mos of treatment with elliptinium.

measurable was the duration of the 44 apparent responses: > 6 months (39 patients) and > 12 months (five).

Toxic Effects

The side effects of elliptinium are summarized in table 3. We confirmed the findings reported in our previous papers as well as in papers published by other groups (7,8,11,12,14). These complications were the same in our series of osseous metastases and our overall series of 328 patients.

Hematologic toxicity.—The lack of marrow toxicity appears to be the most important characteristic of elliptinium. In fact, patients with leukopenia of < 1800 wbcs/mm^3 or thrombocytopenia of < 20,000 platelets/

TABLE 2.—Objective response to elliptinium in osseous metastases from breast cancer, in relation to the place of this compound in the therapeutic sequence*

Series	Therapeutic sequence	No. of patients	No. of responses (%)	Remission		Comments
				Duration (mos)	No. of patients	
1	Elliptinium as last treatment after hormonal procedures	69	19 (27)	3–6	2	In 4 patients, elliptinium was discontinued before the 6th infusion, because of intolerance; they were considered as failures
				6–9	6	
				9–12	9	
				> 12†	2	
2	Elliptinium as 1st systemic treatment after hormonal procedures	66	25 (37)	3–6	3	In 3 patients, elliptinium was discontinued before the 6th infusion, because of intolerance; they were considered as failures
				6–9	7	
				9–12	12	
				> 12‡	3	
						In 4 of the 25 patients, when response ceased, further remission was obtained with conventional chemotherapy
1 and 2		135	44 (32)			

*All patients were previously treated with hormonal procedures except when estrogen receptors were known to be absent.
†12–15 mos.
‡12–17 mos.

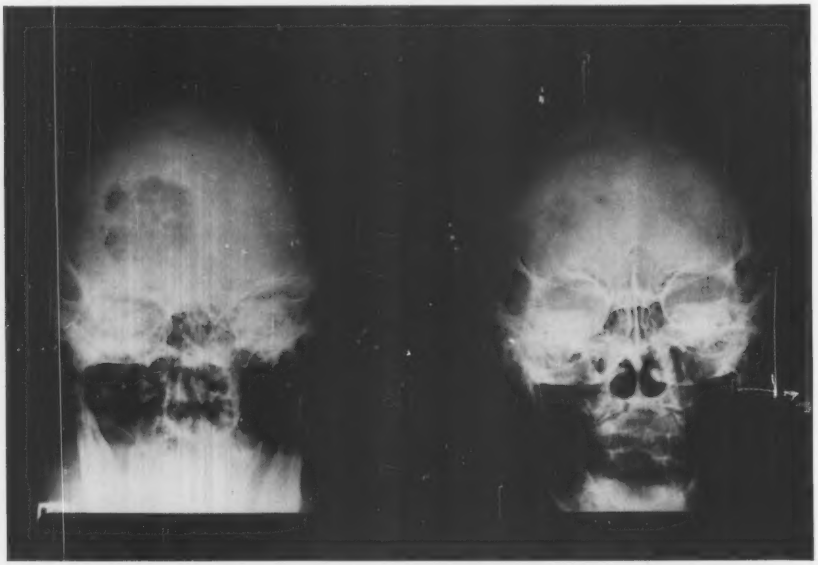


FIGURE 3.—Objective response after 10 mos of treatment with elliptinium (same patient as fig 2).

FIGURE 4.—Objective response after 9 mos of treatment with elliptinium (in the sella turcica are yttrium seeds and tantalum screw positioned during hypophysiolysis performed in Nov 1975).

mm^3 were able to undergo several months of treatment without aggravation of their marrow hypoplasia. This unusual tolerance can, however, only be affirmed under the conditions of our study, ie, at doses of 100 mg/m^2/week. Brugarolas et al (14) reported that patients receiving weekly doses of 100–160 mg/m^2 had a slight tendency to develop leukopenia and thrombocytopenia. Whatever it may be, the drug's lack of hematologic toxicity was found to be especially valuable in patients with osseous metastases from breast cancer, where frequent marrow involvement makes it difficult to administer the usual cytostatic agents.

Renal toxicity.—This complication was not reported in phase I studies of the drug (8,9). Irreversible renal insufficiency developed in four patients, whose treatment was continued after they presented abnormal creatinine levels, at respective cumulative doses of 2400, 2550, 2950, and 4000 mg/m^2. Biopsies in three of these patients showed that the renal insufficiency might be a consequence of tubular lesions; the glomeruli were intact. These renal disorders could not have resulted from hypercalcemia, a frequent complication of bone metastases, since the blood calcium level was normal in these four patients. Investigations are underway to determine whether a lower dose (< 100 mg/m^2/week) combined with a fluid supplement might decrease these drug-induced nephrotoxic effects.

Asialia.—Paradoxically, dryness of the mouth, an apparently minor complication, often constituted the most serious obstacle to routine continuation of treatment. Three-fourths of the patients developed asialia after 6–8 weeks of treatment. This uncomfortable effect might also be the primary cause of other complications observed: oropharyngeal mycosis, anorexia, asthenia, weight loss, etc. The use of an effective artificial saliva (trials are currently underway) would considerably facilitate the continuation of treatment with elliptinium.

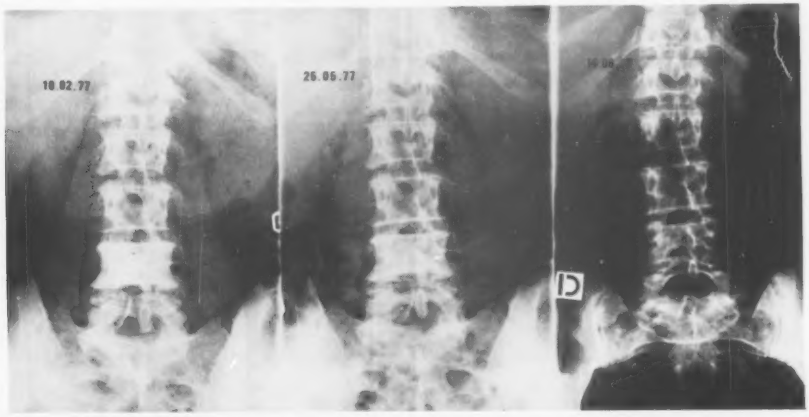


FIGURE 5.—Objective response after 4 mos of treatment in a patient with an osteoblastic metastasis.

DISCUSSION

The principal interest of elliptinium lies in the following two characteristics. (*a*) This compound is able to induce important remissions in bone metastases from breast cancer, and (*b*) its marrow toxicity (if any) is very low, with the therapeutic dosages used in this study. These characteristics are especially interesting since bone marrow involvement is frequent in cases of bone metastases and because the use of most chemical drugs is limited by their toxic effect on the bone marrow.

At the present time, the use of elliptinium in bone metastases from breast cancer raises the following two main problems.

(*a*) *What is the optimal dosage?*—In the first phase I studies (8,9), 80–100 mg/m²/week appeared to be the most satisfactory dose. Since there was late nephrotoxicity, lower routine doses may be more suitable. With regard to the asialia, a trial is underway to see whether infusions on 2 consecutive days with intervals of 2 weeks, or on 3 consecutive days with intervals of 3 weeks, would obtain the same therapeutic effect with less unpleasant side effects.

(*b*) *What is the optimal place of elliptinium in the management of bone metastases from breast cancer?*—As stated earlier, first preference is given at the CFB to hormonal treatments, and chemical cytostatics are used after effects of the hormonal treatments wear off. Should elliptinium be given as the first chemical treatment, followed after failure by conventional cytostatics, or should the order be reversed? Our two nonrandomized series, comprising 66 and 69 patients, respectively, are too small to draw comparative conclusions. A randomized study has just been started in an attempt to compare the two protocols according to the number of remissions obtained, length of remissions, survival time, and discomfort of the patients.

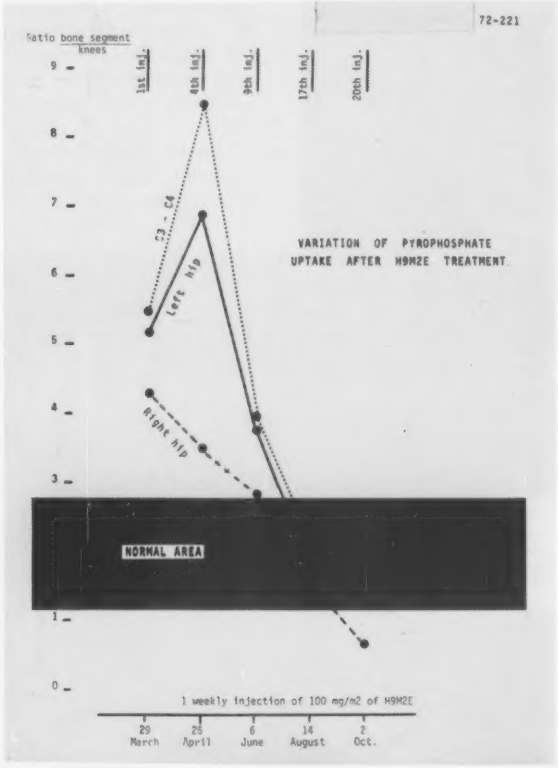


FIGURE 6.—Objective response after 8 mos of elliptinium, expressed by a normalization of the ^{99m}Tc pyrophosphate scintigram, quantified in terms of: $\frac{\text{uptake on metastatic segments}}{\text{uptake on normal bone}}$.

TABLE 3.—Toxic effects and intolerance disorders resulting from elliptinium (100 mg/m²/wk) in 135 patients with bone metastases from breast cancer

Effects	No. of patients (%)	Comments
Digestive disorders		
Nausea	135 (100)	
Vomiting (usually slight)	103 (76)	In 7 patients, treatment had to be discontinued before the 6th infusion, because of severe vomiting
Marrow toxicity	0	In 99 patients with > 4000 wbcs/mm³, no tendency toward leukopenia. In 36 patients with < 4000 wbcs/mm³, no aggravation of the hypoplasia was observed. In 5 patients with < 1800 wbcs/mm³ (platelets < 20,000/mm³ in 2 cases), treatment could be continued from 7 to 29 infusions without worsening of these disorders
Liver toxicity	0	
Renal toxicity	8*	Irreversible insufficiency was observed in 4 patients who had received > 24 infusions
Immunologic disorders	35 (26)†	Hemolysis was observed in 3 cases; hemodialysis was necessary in 1 case
Asialia	101 (75)	
Tongue mycosis	10 (7)	
Fever	6 (4)	5 cases < 39°C, 1 case of 40°C, lasting not longer than 24 hrs
Cramps	32 (25)‡	Usually attenuated by quinine benzoate or amiodarone
Asthenia	§	
Anorexia	§	

*Rise in blood creatinine observed after 20 weekly infusions.
†Appearance of anti-ellipticine antibodies.
‡After 20 weekly infusions.
§Usual after 20 weekly infusions.

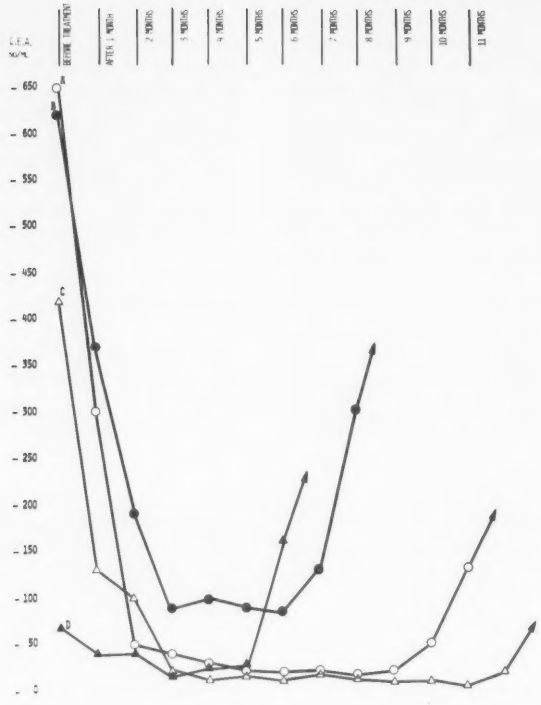


FIGURE 7.—Objective remissions expressed by the normalization of the serum CEA in 4 women with osseous metastases treated with elliptinium. Duration of remissions: 9 mos (patient A); 6 mos (patient B); 11 mos (patient C); and 5 mos (patient D).

REFERENCES

1. DALTON LK, DEMERAC S, ELMES BG, ET AL. Synthesis of the tumour-inhibitory alkaloids, ellipticine, 9-metho-ellipticine and related pyrido (4-3 b) carbazoles. Aust J Chem 20:2715–2727, 1967.
2. LE PECQ JB, DAT-XUONG N, GOSSE C, ET AL. A new antitumoral agent: 9-hydroxyellipticine. Possibility of a rational design of anticancerous drugs in the series of DNA intercalating drugs. Proc Natl Acad Sci USA 71:5078–5079, 1974.
3. LE PECQ JB, GOSSE C, DAT-XUONG N, ET AL. Deux nouveaux dérivés anti-tumoraux: l'hydroxy-9-methyl-2-ellipticinium (acétate) et l'hydroxy-9-dimethyl-2,6 ellipticinium (chlorure). Action sur la leucémie L 1210 de la souris. C R Acad Sci (Paris) 281:1365–1367, 1975.
4. LE PECQ JB, GOSSE G, DAT-XUONG N, ET AL. Antitumor activity of 9-hydroxyellipticine (NSC 210 707) on leukemia and the effect of route of injection. Cancer Res 36:3067–3076, 1976.
5. NSARI BM, and THOMPSON EN. Methoxy-9-ellipticine lactate in refractory acute myeloid leukemia. Postgrad Med J 51:103–105, 1975.
6. MATHÉ G, HAYAT M, DE VASSAL F, ET AL. Methoxy-9-ellipticine lactate. III. Clinical screening: its action in acute myeloblastic leukemia. Rev Eur Etud Clin Biol 15:541–545, 1970.
7. JURET P, LE TALAER JY, COUETTE JE ET AL. Hydroxy-9-methyl-2-ellipticinium (NSC 264-137) in 52 cases of osseous metastases from breast cancer. *In* Breast Cancer: Experimental and Clinical Aspects (Mouridsen HT, and Palshof T, eds). Oxford, England, Pergamon Press, 1980, pp 277–279.
8. JURET P, TANGUY A, GIRARD A, ET AL. Preliminary trial of 9-hydroxy-2-methyl-ellipticinium (NSC 264-137) in advanced human cancers. Eur J Cancer 14:205–206, 1978.
9. JURET P, TANGUY A, GIRARD A, ET AL. L'acétate d'hydroxy-9-methyl-2-ellipticinium (NSC 264–137). Etude toxicologique et thérapeutique chez 100 cancéreux. Nouv Presse Med 8:1495–1498, 1979.
10. JURET P, TANGUY A, GIRARD A, ET AL. Action de l'hydroxy-9-methyl-2-ellipticinium dans les métastases osseuses du cancer du sein. J Med Caen 13:61–66, 1978.
11. CLARYSSE A, BRUGAROLAS A, SIEGENTHALER P, ET AL. 9-Hydroxy-2*N*-methyl ellipticinium (NSC 264–137) in advanced breast cancer. A report of the Early Clinical Trial Group of the EORTC. Eur J Cancer, 1981 (in press).
12. ROUESSE J, TURSZ T, LE CHEVALIER T, ET AL. Intérêt de la 2*N*-méthyl-9-hydroxy-ellipticine (NSC 264–137) dans le traitement des cancers métastasés. Nouv Presse Med 10:1997–1999, 1981.
13. ROBILLARD J, COUETTE JE, LY VH, ET AL. La scintigraphie numérique dans l'exploration des métastases osseuses. J Fr Biophys Med Nucl 5:207–213, 1977.
14. BRUGAROLAS A, GARCIA M, DE JAGER R, ET AL. 9-Hydroxy-methyl-ellipticinium: phase I clinical study. Proc Am Assoc Cancer Res and ASCO 20:310, 1979.

Results of Aggressive Chemotherapy for Acute Nonlymphoblastic Leukemia in Adults[1,2]

James O. Armitage,* C. Patrick Burns, Fred R. Dick, Suzanne B. Aunan, and Donald J. Slymen[3,4]

Sixty-five patients with acute nonlymphoblastic leukemia, ranging in age from 16 to 73 years (median, 54), were treated with thioguanine, cytarabine, daunorubicin, prednisone, and vincristine to induce complete remission. Remission was maintained with monthly 5-day courses of cytarabine and thioguanine for 4 years. Of the entire group, 57% achieved complete remission and 29% died in the first 30 days of treatment. The median remission duration was 15 months, with 20% of the complete responders predicted to remain in complete remission for 4 years. No primary central nervous system relapses have occurred. Of six variables examined by multivariate analysis, only age was predictive of remission success. Before analysis, the patients were divided into groups by age: *group 1*—ages 16–42 years (n = 17); *group 2*—ages 43–59 years (n = 25); and *group 3*—ages 60–73 years (n = 23). Complete remission rates were 76% in group 1, 64% in group 2, and 35% in group 3 (P = 0.002). Lower remission rates for older patients resulted from more early deaths rather than resistant disease. Age and LDH levels affected remission duration. The median complete remission duration was 48 months in group 1 and 10 months in groups 2 and 3 (P = 0.004). In group 1, 41% of patients achieving complete remission are predicted to remain in complete remission for 4 years. We conclude that this five-drug induction regimen is highly effective for induction therapy in younger adults with acute nonlymphoblastic leukemia. The results in patients ≥ 60 years of age are less satisfactory due to a lower remission rate and greater chance of early death. Furthermore, these results demonstrate that the program as described results in a longer duration of complete remission for patients of younger age. [Cancer Treat Rep 66:1917–1924, 1982]

Intensive combination chemotherapy in children with acute lymphoblastic leukemia has been successful in producing a cure in approximately one-half of the cases where it has been applied (1). Although similarly aggressive treatment in adults with acute nonlymphoblastic leukemia (ANLL) unfortunately has not met with the same success, recent reports have described improvement in the results of treating ANLL (2,3). Previous series have often contained a predominance of pediatric patients or had a younger median age than is characteristic for adult ANLL in general. Whether or not age is a prognostic factor in the treatment of ANLL has been a point of controversy. If older age is an adverse prognostic factor, it would be important to identify the reasons, and if possible, modify management accordingly. In addition, bone marrow transplantation after high-dose chemotherapy and total-body irradiation has recently been demonstrated to be effective "maintenance" or "consolidation" therapy in children and young adults with ANLL (4–6).

Since 1975, we have treated 65 adults with ANLL using an intensive remission-induction regimen of

[1]Received Mar 25, 1982; revised July 23, 1982; accepted Aug 5, 1982.

[2]Supported in part by the Iowa Leukemia and Cancer Research Fund. Dr. Burns is the recipient of Public Health Service Research Career Development Award CA-00324 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medicine (*J. O. Armitage, C. P. Burns,* and *S. B. Aunan*), Pathology (*F. R. Dick*), and Preventive Medicine and Environmental Health (Biostatistics Section) (*D. J. Slymen*), University of Iowa College of Medicine, Iowa City.

[4]We gratefully acknowledge the aid and support in patient care provided by the nurses and house staff of the University of Iowa Hospitals.

**Reprint requests to:* James O. Armitage, MD, Department of Medicine, University of Nebraska Medical Center, 42nd and Dewey Ave, Omaha, NE 68105.

thioguanine, cytarabine, daunorubicin, prednisone, and vincristine and then maintaining complete responses with monthly 5-day courses of cytarabine and thioguanine for 4 years. Preventive central nervous system (CNS) therapy was not given. In presenting the results of this treatment regimen, we describe the complete remission rate, the chances for long-term disease-free survival, the frequency of CNS relapse, and the effect of age and other prognostic factors on treatment outcome.

PATIENTS AND METHODS

Patient Characteristics

The patients in this study were first treated for ANLL at the University of Iowa Hospitals between February 1975 and June 1980. All patients treated with this regimen through June 1980 are included. The patients varied in age from 16 to 73 years (median, 54). The age limits of the three age groups used in analyzing the data (table 1) were established prior to any analysis. Sixty years has been frequently used in other studies. Patients < 60 years old were further divided at 42 years, since this is the age cutoff we have used for bone marrow transplantation at our institution. None of the patients had received previous antileukemia therapy. The subtype of acute leukemia and hematologic parameters for the patients are presented in table 1. ANLL was subclassified using French-American-British (FAB) criteria (7).

Complete remission was diagnosed when there were < 5% blasts or 10% blasts plus promyelocytes in a normally cellular marrow, along with a granulocyte count of at least 1.5 × 10^9/L, a platelet count of at least 100 × 10^9/L, and a rising hemoglobin level. Bone marrow was tested monthly before each course of maintenance chemotherapy, and relapse was diagnosed when the blasts were definitely > 5% or the blasts plus promyelocytes were > 10% of the marrow nucleated cells. Complete remission duration was defined as the time from diagnosis of complete remission to the identification of relapse, which was almost always when the patient was asymptomatic. Survival was defined as the time from beginning of chemotherapy to death. For purposes of this report, early death was defined as a death, almost always from infection, within 30 days of starting induction chemotherapy. The diagnosis of CNS leukemia was considered established when leukemic cells were identified in the cerebrospinal fluid. Routine cerebrospinal fluid examinations were not performed. However, lumbar punctures were performed whenever the clinical symptoms and signs were suggestive of CNS leukemia. Since August 1976, all cerebrospinal fluid examinations have included the evaluation of smears prepared using a cytocentrifuge technique.

TABLE 1.—Patient characteristics

Characteristic	Age group 1	2	3	All patients
Age (yrs)	16–42	43–59	60–73	16–73
No. of patients	17	25	23	65
Male:female	7:10	18:7	12:11	37:28
FAB subtype				
M1	4	6	7	17
M2	4	8	7	19
M3	2	1	1	4
M4	6	8	4	18
M5	1	1	4	6
M6	0	0	0	0
RAEB*	0	1	0	1
Wbc count (× 10^9/L)				
Median	26.6	8.5	3.8	
Mean	44.7	21.8	25.0	
Platelet count (× 10^9/L)				
Median	45.0	26.0	55.0	
Mean	114.9	55.5	88.7	
LDH level (IU/L)				
Median	606.0	472.0	318.0	
Mean	681.6	686.2	355.0	

*Diagnosed early in study as erythroleukemia then reclassified as refractory anemia with excess blasts (RAEB).

Treatment Regimen

The treatment regimen was as follows:

Induction*:	Thioguanine, 75 mg/m^2 orally q 12 hrs, Days 1–7 Cytarabine, 75 mg/m^2 iv q 12 hrs, Days 1–7 Daunorubicin, 60 mg/m^2 iv, Days 1–3 Prednisone, 40 mg/m^2 orally, Days 1–7 Vincristine, 1 mg/m^2 iv, Days 1 and 7
Consolidation:	Thioguanine, 75 mg/m^2 orally q 12 hrs, Days 1–5 Cytarabine, 75 mg/m^2 iv q 12 hrs, Days 1–5 Daunorubicin, 60 mg/m^2 iv, Days 1–2 Prednisone, 40 mg/m^2 orally, Days 1–5 Vincristine, 1 mg/m^2 iv, Day 1
CNS prophylaxis: None	
Maintenance:	Cytarabine, 100 mg/m^2 iv q 12 hrs × 5 days Thioguanine, 100 mg/m^2 orally q 12 hrs × 5 days, repeat q 4–5 wks

*Repeated on Day 15 if leukemia persisted in marrow.

All patients received the first 7-day sequence after pretreatment with allopurinol. A bone marrow biopsy was performed 7 or 8 days after completing the first 7 days of chemotherapy, and a treatment decision was based on marrow cellularity. If the marrow was hypocellular, further treatment was withheld until recovery occurred. If the marrow remained cellular (ie, $\geq$ 30% cellularity) with persistent leukemia, the 7-day treatment sequence was repeated. In the very unusual case of a cellular marrow with normal maturation, treatment was withheld. As soon as the patient met the criteria for complete remission, the 5-day consolidation sequence utilizing the same agents was administered. Then, when marrow and peripheral blood recovery was evident, the maintenance regimen was begun and administered at 4- or 5-week intervals for 4 years. Blood cell counts were routinely performed only at the initiation of each maintenance sequence. There was no significant difference in dose adjustments among the different age groups. After 4 years of continuous remission, all treatment was discontinued and the patients were followed with intermittent bone marrow examination. Patients were uniformly hospitalized during the consolidation sequence. Maintenance chemotherapy was administered on an outpatient basis, with the iv cytarabine given in a clinic, emergency room, or, rarely, by a family member or neighbor. Post-relapse therapy was individualized.

Statistical Methods

All patients in this study were considered evaluable. Complete remission duration and survival of groups were calculated using the life table method. Factors influencing survival time and complete remission duration were analyzed using the proportional hazards model. The model may be stated in terms of the hazard function, $\lambda(t)$, which for survival time may be defined as the probability that an individual will die in a short time interval, given survival to the beginning of the interval. The hazard function may be expressed for an individual as:

$$\lambda(t) = \exp\{X'\beta\}\lambda_0(t),$$

where $\lambda_0(t)$ is the underlying hazard for all individuals, X is a p-vector of observed values on an individual for the set of p factors we wish to examine, and β is a p-vector of unknown regression coefficients which will be estimated. This method is detailed in the report of Cox (8); a simpler approach to the estimation scheme, which was employed in this analysis, was derived by Breslow (9).

The analysis of factors influencing achieving complete remission was accomplished using a multiple logistic model. It is assumed that the probability of achieving complete remission is $\exp\{-X'\beta\}/(1 + \exp\{-X'\beta\})$, where X is the vector of observed values and β is the vector of regression parameters to be estimated. Six variables were examined: age group (1 = 16–42, 2 = 43–59, and 3 = 60–73), wbc count, platelet count, serum LDH level, sex, and subtype of ANLL (combined M1, M2, and M3 versus combined M4 and M5). The one patient with RAEB (table 1) was excluded from this analysis. In addition, age without grouping was examined. Table 1 displays the mean and median wbc and platelet counts and LDH levels by age group. For the 64 patients included in the analyses, 13 had censored survival times. An equivalent sample size with no censoring is 58. Complete remission was achieved for 36 of the patients included in the analyses. Of these, 12 had censored remission duration times; an equivalent sample size with no censoring is 29.

Multivariate analyses examined the simultaneous effects of the set of risk factors on survival time, complete remission, and remission duration. For each analysis, a backward elimination procedure was used to remove from the model variables that did not contribute additional significant information to the model beyond the set of factors included. The variable with the smallest chi-square statistic was removed at each step, provided the associated P value was > 0.05. The analyses are reported using the age grouping factor instead of age. However, when the actual age values were used, the results from the backward elimination method were substantially the same. The significance of differences between groups that were not part of the multivariate analysis was determined using Fisher's exact test.

RESULTS

Thirty-seven of the 65 patients (57%) in this series achieved complete remission. The chances for complete

TABLE 2.—Results of treatment by age group

Characteristic	Age group 1	2	3	All patients
Complete remissions (CR)(%)*	76	64	35	57
Early deaths (ED)(%)†	6	24	52	29
CR + ED (%)	82	88	87	86
Median CR duration (mos)‡	48	10	10	15
CNS relapses	0	0	0	0
Predicted 4-yr CR (%)	41	8	17	20
Overall median survival (mos)§	27	9	< 1	9

*1 + 2 > 3, $P = 0.002$.
†1 < 2 + 3, $P = 0.015$.
‡1 > 2 + 3, $P = 0.004$.
§1 > 2 + 3, $P < 0.001$.

remission varied by age, being 76% in group 1, 64% in group 2, and 35% in group 3 (table 2). Multivariate analysis identified only age (ie, either by age group or individually) as significantly associated with chances to achieve a complete remission. Patients in groups 1 and 2 were significantly more likely to achieve complete remission than those in group 3 ($P = 0.002$). Sex, wbc count, platelet count, subtype of ANLL, and LDH level did not affect the chances of achieving a complete remission. The frequency of early deaths (ie, within 30 days of the beginning of therapy) and complete remission rate were inversely related, and the sum of the complete remission rate and the early death rate was relatively constant between groups. Therefore, any variation among age groups in the proportion of patients with chemotherapy-resistant disease could not explain the differences in outcome. Early deaths occurred in 6% of the patients in group 1, in 24% of the patients in group 2, and in 52% of the patients in group 3. Patients in groups 2 and 3 were significantly more likely to die in the first 30 days of therapy than those in group 1 ($P = 0.015$).

Twenty percent of the entire group of 37 patients achieving complete remission are predicted to remain in remission at 4 years, with a median complete remission duration of 15 months (fig 1). The duration of complete remission, once achieved, is presented by age group in figure 2. Eight of 13 complete responders in group 1, three of 16 in group 2, and two of eight in group 3 remain in complete remission from 5+ to 69+ months

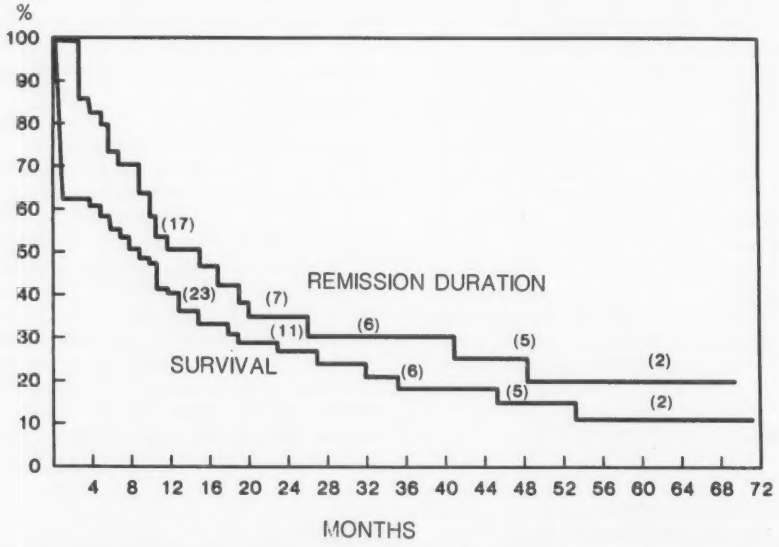


FIGURE 1.—Survival from initiation of therapy for the entire group of 65 patients, and complete remission duration for the 37 patients achieving remission. Values in parentheses = No. of patients surviving or remaining in remission to the indicated interval.

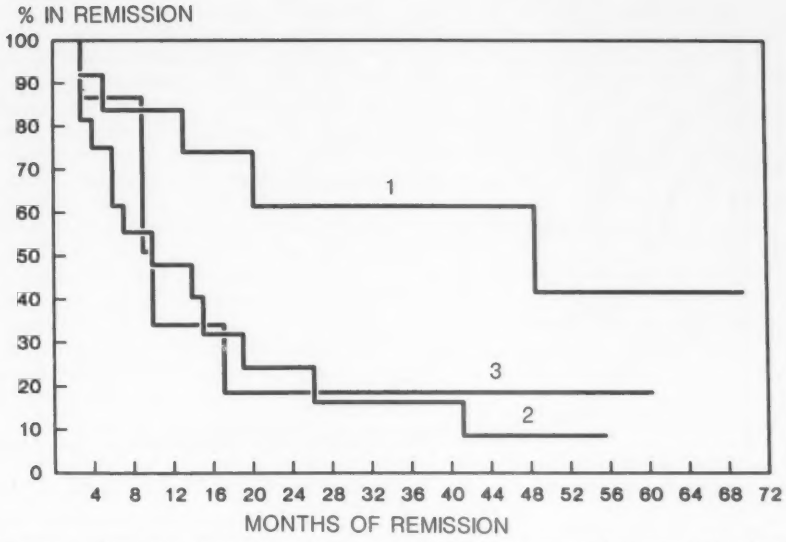


FIGURE 2.—Complete remission duration for patients in the following age groups: 16–42 (group 1, 13 patients), 43–59 (group 2, 16 patients), and 60–73 (group 3, 8 patients). Patients in group 1 had significantly longer remissions than patients in groups 2 and 3 ($P = 0.004$).

(median, 24+). The median complete remission duration was 48 months in group 1, 10 months in group 2, and 10 months in group 3 (table 2). Forty-one percent of patients in group 1 are predicted to be in complete remission at 4 years, in contrast to 8% of patients in group 2 and 17% of patients in group 3. Patients in group 1 had significantly longer remissions than those in groups 2 and 3 ($P = 0.004$). Multivariate analysis identified age group, age, and LDH level as being significantly associated with remission duration. Prolonged remissions were associated with lower levels of LDH ($P = 0.002$). The effects of age and LDH on remission duration were examined in another model and were independent of one another. In fact, younger patients, who had the longest remissions, also had the highest LDH levels ($P = 0.007$), and the effect of LDH on remission duration was evident within each age group. An effect of LDH on remission duration has been previously reported (10). Sex, wbc count, platelet count, subtype of ANLL, and peak SGOT level in the first 2 months of therapy (ie, to estimate the effect of treatment-related hepatitis) did not significantly affect remission duration. There were no primary CNS relapses despite the absence of prophylactic therapy to the CNS. To date, there have been no relapses in four patients followed 1, 8, 13, and 21 months after maintenance treatment was discontinued.

The median survival for the entire group of 65 patients is predicted to be 9 months (fig 1). This varied from 27 months in group 1, to 9 months in group 2, to < 1 month in group 3 (fig 3). Fourteen patients remain alive from 7 to 71 months after beginning therapy. Survival was significantly associated with age (ie, either by age group or individually) and LDH level. The survival of patients in group 1 was significantly longer than for patients in groups 2 and 3 ($P = 0.001$). Lower levels of LDH were associated with prolonged survival ($P = 0.002$). The effects of age and LDH on survival were examined in another model and were independent. Sex, wbc count, platelet count, and subtype of ANLL did not significantly affect survival.

DISCUSSION

In this study we found age-dependent remission rates varying from 76% to 35% and durations of remission from 48 to 10 months. The data demonstrate that the five-drug induction and consolidation program followed by the two-drug maintenance we utilized is effective in the therapy of adult ANLL. The fact that 41% of the younger patients achieving a remission are predicted to remain in remission for 4 years demonstrates that the disease can now be controlled and perhaps cured in an appreciable percentage of patients.

We selected the five-drug induction program for inclusion in our regimen after it was initially reported to have favorable results (11) and had showed promising results in our own preliminary experience as part of a

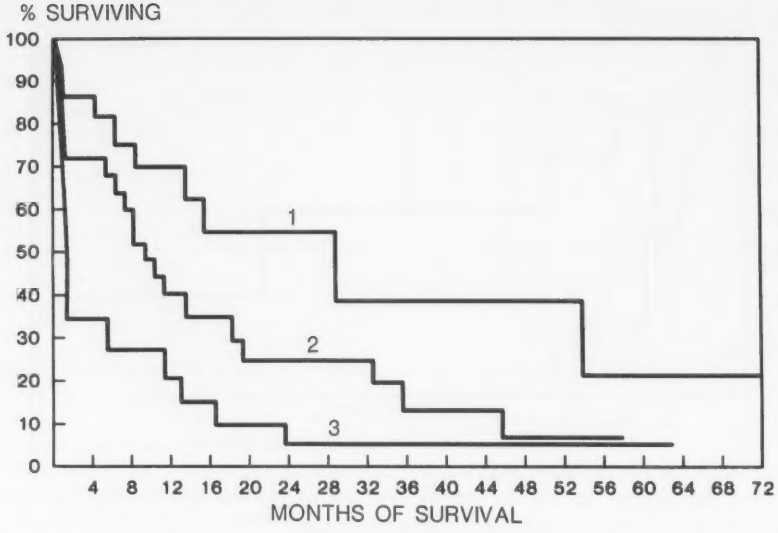


FIGURE 3.—Survival from the initiation of therapy for all treated patients in the following age groups: 16–42 (group 1, 17 patients), 43–59 (group 2, 25 patients), and 60–73 (group 3, 23 patients). Patients in group 1 survived significantly longer than those in groups 2 and 3 ($P < 0.001$).

multi-institutional trial (12). Furthermore, the combination seemed theoretically logical. It seemed clear that daunorubicin and cytarabine, which are the two most active single agents for adult ANLL, should be included in induction programs. The addition of thioguanine seemed justified on the basis of studies in animal models which showed it to be synergistic with cytarabine in experimental systems (13). Vincristine and prednisone have some small single-agent activity (14). We were attracted to a program which included them because of the occasional impreciseness of the differentiation between ANLL and ALL in adults. For example, the L2 lymphoid category of the FAB classification has been considered as undifferentiated or stem cell leukemia and treated as ANLL by many institutions; in one recent series, one-half of these cases exhibited high levels of terminal transferase and a lower remission rate when compared to other myeloid groups (15). The maintenance program was utilized because of our previously reported good results (16) and the desirability of including a drug which crosses into the CNS when administered systemically.

The adverse effect of age on chances to achieve a complete remission from ANLL with chemotherapy has, with a few exceptions (17,18), been a reproducible finding in previous studies. In the present study, age had no statistically significant effect until 60 years. The relatively constant sum of complete remission rate and early death rate in the various age groups in this study suggests that the lower remission rate in older patients is a direct result of their inability to tolerate the period of hypoplasia that is associated with remission induction chemotherapy. Thus, shortening the period of hypoplasia by a modification in therapy or improved supportive measures would seem obvious approaches to this problem. It may be that the older group of patients are the ones most likely to benefit from protected environments such as laminar air flow rooms or prophylactic antibiotics.

Some previous studies have reported that there is no adverse effect of age on duration of remission (19–23). In our study, there was a significantly longer duration of remission for patients < 42 years old compared to those > 42. However, the age groups 43–59 and 60–73 did not differ. Other studies have also reported that duration of remission is inversely related to age (10,24).

Although CNS involvement in adults with ANLL is certainly much less frequent than in children with acute lymphoblastic leukemia, it has been increasingly recognized as a clinical problem and seems to be particularly associated with monocytic subtypes (25). As complete remission durations improve, it might be anticipated that CNS relapse would become more frequent and necessitate attempts at prophylactic CNS therapy. With the treatment regimen we employed, this has not been the case. Meyer et al (25) and Wiernik et al (26) have demonstrated that routine lumbar punctures reveal unexpected CNS leukemia in as many as

18% of newly diagnosed patients. Because routine lumbar punctures were not performed in this study, it is impossible to be certain that subclinical disease was not present in a proportion of patients. However, the absence in all patients of overt CNS leukemia terminating remission makes this unlikely. The low CNS relapse rate that we observed may be related to the inclusion of cytarabine in the maintenance therapy. Cytarabine is known to penetrate the CNS (27), and Herman et al (28) observed a decreased incidence of CNS recurrence in patients with diffuse histiocytic lymphomas when a maintenance regimen including cytarabine was used.

The maintenance treatment that we employed is administered on an outpatient basis and is associated with minimal morbidity and no mortality (16). Similar results using a comparable maintenance regimen have been reported by Peterson and Bloomfield (19). More intensive maintenance regimens and "late intensification" requiring hospitalization and associated with morbidity and risk of death should not be accepted as standard approaches until it is demonstrated that they yield superior survival to effective outpatient maintenance programs.

It is not possible to know whether the successful outcome of our therapeutic program is the result of tumor burden reduction during induction, consolidation, or maintenance. However, there are data to suggest that the maintenance portion is an important component. Glucksberg et al (29) have recently reported remission rates similar to ours using an induction and consolidation program that is identical to ours in drugs and schedule and differing only slightly in doses. In that study, the median remission duration of 39 weeks was considerably less than the 64 weeks in our study. If this difference is real, as it appears to be, the longer duration in our study could have resulted from a more effective maintenance program which differed from theirs in the dose and rate of cytarabine and because they also utilized prednisone and vincristine. Furthermore, for most of their patients maintenance therapy was discontinued at 30 months.

Several recent studies besides our own have demonstrated improved results in the chemotherapy of ANLL (3,19,29–31). Complete remission rates as high as 85% (32) and median remission durations with chemotherapy for all patients of as long as 27 months (33) have been reported. It has become apparent that a proportion of patients can have long-term disease-free survival after chemotherapy has been discontinued (30). A small but real proportion of adults with ANLL can be cured using currently available chemotherapy. Bone marrow transplantation after high-dose chemotherapy and total-body irradiation when applied to patients with ANLL in remission has been reported to yield relapse-free survival in > 60% of patients with observation as long as 3 years (4). However, bone marrow transplantation is limited by age and by the fact that approximately 60% of potential candidates lack a donor. Children tolerate bone marrow transplantation better than adults, and few successful transplants have been performed after the age of 40. Whether a similar effect of age on survival in complete remission is operative in patients treated with maintenance chemotherapy has been a point of controversy, as previously discussed. In this study, we found a long remission duration in adults in the age group that our institution has considered candidates for bone marrow transplantation. These results emphasize the need for a prospective clinical trial comparing bone marrow transplantation with an effective maintenance chemotherapy regimen in young adults.

REFERENCES

1. Pinkel D. The ninth annual David Karnofsky lecture: Treatment of acute lymphocytic leukemia. Cancer 43:1128–1137, 1979.
2. Bloomfield CD. Treatment of adult acute nonlymphocytic leukemia—1980. Ann Intern Med 93:133–135.
3. Weinstein JH, Mayer RJ, Rosenthal DS, et al. Treatment of acute myelogenous leukemia in children and adults. N Engl J Med 303:472–478, 1980.
4. Thomas ED, Buckner CD, Clift RA, et al. Marrow transplantation for acute nonlymphoblastic leukemia in first remission. N Engl J Med 301:597–599, 1979.
5. Powles RL, Morgenstern G, Clink HM, et al. The place of bone marrow transplantation in acute myelogenous leukemia. Lancet 1:1047–1150, 1980.
6. Blume KG, Beutler E, Cross KJ, et al. Bone marrow ablation and allogenic marrow transplantation in acute leukemia. N Engl J Med 302:1041–1046, 1980.
7. Bennett JM, Catovsky D, Daniel M-T, et al. Proposals for the classification of the acute leukemias Br J Haematol 33:451–458, 1976.
8. Cox DR. Regression models and life tables. J R Stat Soc B34:187–208, 1972.
9. Breslow N. Covariance analysis of censored survival data. Biometrics 30:89–99, 1974.
10. Keating, MJ, Smith TL, Gehan EA, et al. Factors related to length of complete remission in adult acute leukemia. Cancer 45:2017–2029, 1980.
11. Glucksberg H, Buckner CD, Fefer A, et al. Combination chemotherapy for acute nonlymphoblastic leukemia in adults. Cancer Chemother Rep 59:1131–1137, 1975.
12. Greenberg B, Lewis J, Burns CP, et al. A re-evaluation of intensive induction therapy in adult acute nonlymphatic leukemia. Proc Am Assoc Cancer Res and ASCO 17:268, 1976.
13. Schabel FM, Jr. Synergism and antagonism among antitumor agents. *In* Pharmacological Basis of Cancer Chemotherapy. Baltimore, Williams and Wilkins, 1975, pp 595–623.
14. Goldin A, Sandberg JS, Henderson ES, et al. The chemotherapy of human and animal acute leukemia. Cancer Chemother Rep 55:309–507, 1971.
15. Mertelsmann R, Thaler HT, To L, et al. Morphological classification, response to therapy, and survival in 263 adult patients with acute nonlymphoblastic leukemia. Blood 56:773–781, 1980.
16. Armitage JO, and Burns CP. Maintenance therapy of adult acute nonlymphoblastic leukemia. Cancer 41:697–700, 1977.
17. Gale RP, and Cline MJ. High remission induction rate in acute myeloid leukemia. Lancet 1:497–499, 1977.

18. Peterson BA, Bloomfield CD, Bosl GJ, et al. Intensive five-drug combination chemotherapy for adult acute nonlymphocytic leukemia. Cancer 46:663–668, 1980.
19. Peterson BA, and Bloomfield CD. Prolonged maintained remissions of adult acute nonlymphoblastic leukemia. Lancet 2:158–160, 1977.
20. Peterson BA, and Bloomfield CD. Treatment of acute nonlymphocytic leukemia in elderly patients. A prospective study of intensive chemotherapy. Cancer 40:647–652, 1977.
21. Henderson ES, Wallace JH, Yates J, et al. Factors influencing prognosis in adult acute myelocytic leukemia. Adv Biosci 14:71–82, 1975.
22. Weil M, Jacquillat CI, Gemon-Anclerc MF, et al. Acute granulocytic leukemia. Treatment of the disease. Arch Intern Med 136:1389–1395, 1976.
23. Gale RP, Foon KA, Cline MJ, et al. Intensive chemotherapy for acute myelogenous leukemia. Ann Intern Med 94:753–757, 1981.
24. Enck RE, Bauman AW, and Bennett JM. Adult acute leukemia. The Rochester (NY) Experience. Arch Intern Med 136:1256–1261, 1976.
25. Meyer RJ, Ferreira PPC, Cuttner J, et al. Central nervous system involvement at presentation in acute granulocytic leukemia. A prospective cytocentrifuge study. Am J Med 68:691–694, 1980.
26. Wiernik PH, Schimpff SC, Schiffer CA, et al. Randomized clinical comparison of daunorubicin (NSC-82151) alone with a combination of daunorubicin, cytosine arabinoside, (NSC-63878), 6-thioguanine (NSC-752), and pyrimethamine (NSC-3061) for the treatment of acute nonlymphocytic leukemia. Cancer Treat Rep 60:41–53, 1976.
27. Ho DHW. Potential advances in the clinical use of arabinosyl cytosine. Cancer Treat Rep 61:717–722, 1977.
28. Herman TS, Hammond N, Jones SE, et al. Involvement of the central nervous system by non-Hodgkin's lymphomas. Cancer 43:390–397, 1979.
29. Glucksberg H, Cheever MA, Farewell VT, et al. High-dose combination chemotherapy for acute nonlymphoblastic leukemia in adults. Cancer 48:1073–1081, 1981.
30. McCredie KB, Keating MJ, Dicke DA, et al. Treatment of adult acute myeloblastic leukemia. Haematol Blood Transfus 23:89–92, 1977.
31. Vaughan WP, Karp JE, Burke PJ, et al. Long chemotherapy-free remissions after single-cycle timed-sequential chemotherapy for acute myelocytic leukemia. Cancer 45:859–865, 1980.
32. Rees JKH, Sandler RM, Challener J, et al. Treatment of acute myeloid leukemia with a triple cytotoxic regime: DAT. Br J Cancer 36:770–776, 1977.
33. Presant CA, Berger NA, Klahr C, et al. Remission maintenance for acute nonlymphocytic leukemia. Cytosine arabinoside plus 6-thioguanine versus a sequence of drug regimens. Cancer 46:22–28, 1980.

Use of Methotrexate and 5-FU for Recurrent Head and Neck Cancer[1]

Charlotte Jacobs[2,3,*]

Thirty patients with recurrent squamous cell carcinoma of the head and neck were treated with methotrexate (250 mg/m²) followed 1 hour later by 5-FU (600 mg/m²). One patient had a complete response and four had partial responses, for an overall response rate of 16%. The response rate in patients with no prior chemotherapy was 21%. Toxic effects included neutropenia in ten patients, with one associated death from infection; severe mucositis in three patients; and severe diarrhea in seven patients. Three patients had acute abdominal pain requiring hospitalization, with one associated death. The combination of methotrexate and 5-FU as given in our study was not superior to methotrexate alone and had substantial toxicity. [Cancer Treat Rep 66:1925–1928, 1982]

The role of chemotherapy in the management of recurrent squamous cell cancers of the head and neck has been limited (1–3). Methotrexate, the most extensively studied drug, has reported response rates from 8% to 50%, but most responses are partial and of short duration (4). Although combination chemotherapy has been reported to have higher response rates (5–7), there is often associated increased toxicity. Only one randomized study has shown improvement in response rates with a combination of agents (cisplatin, methotrexate, and bleomycin) compared to methotrexate alone (8).

Pitman et al (9) have described a highly effective regimen of sequential methotrexate plus 5-FU in which treated patients with squamous cell cancer of the head and neck had a 40% complete response rate. This regimen was based on laboratory studies by Cadman et al (10) in which they showed that exposure of L1210 leukemia cells first to methotrexate and then to 5-FU produced a synergistic effect on cell kill.

The purpose of this study was to investigate further the efficacy and toxicity of this regimen for recurrent squamous cell cancers of the head and neck.

PATIENT SELECTION AND METHODS

Between July 1980 and May 1982, 30 patients with recurrent squamous cell carcinoma of the head and neck were treated with moderate-dose methotrexate and 5-FU. All patients had recurrent disease following surgery and/or radiation therapy, and 16 patients had had prior chemotherapy, predominantly with single-agent cisplatin.

Pretreatment evaluation included a complete history and physical examination, cbc, serum creatinine, urinalysis, BUN, liver enzymes, chest X-ray, and appropriate radiographs and scintiscans for measurement of disease. Cbc and serum creatinine were measured prior to each treatment.

Methotrexate (250 mg/m²) was infused over 30 minutes and was followed 1 hour later by 5-FU (600 mg/m²). The patient was prehydrated with 1 L of 5% dextrose in water plus 100 mEq of sodium bicarbonate, and 1 L of the same iv fluid was infused over 2–4 hours following methotrexate. At 24 hours, patients began leucovorin orally: 25 mg initially, then 10 mg every 6 hours for six doses.

Response was evaluated prior to each treatment. A complete response was defined as the complete disappearance of all clinically detectable disease. A partial response was defined as a 50% reduction in the sum of the products of the longest diameters of all measurable lesions without the appearance of new lesions. Duration of response was calculated from the start of response to disease progression. Survival time was calculated from study entry to death. Toxicity was graded according to Northern California Oncology Group Toxicity criteria.

[1]Received June 14, 1982; accepted Aug 24, 1982.

[2]Division of Oncology, Department of Medicine, Stanford University School of Medicine, CA.

[3]I thank Drs. Peter Eisenberg, George Waltuch, and Michael Alexander for clinical material.

*Reprint requests to: Charlotte Jacobs, MD, Division of Oncology, Department of Medicine, Stanford University Medical Center, Rm C005, Stanford, CA 94305.

RESULTS

Patient Characteristics

There were 21 males and nine females, with a mean age of 56 years (27–76). The mean Karnofsky performance status was 80 (range, 40–100). The primary sites of disease were as follows: oropharynx, nine patients; oral cavity, six; larynx, five; nasopharynx, four; hypopharynx, three; sinuses, two; and unknown primary, one. Twenty-three patients had local disease recurrence, five had distant metastases, and two had local disease plus distal metastases. All patients had had prior treatment: 20 had had surgery plus radiation therapy, ten had received radiation therapy, and 16 had had chemotherapy. All patients had squamous cell carcinoma, and the histologic differentiation was determined in 26: moderately differentiated, 12 patients; poorly differentiated, seven; well-differentiated, six; and undifferentiated, one. The mean number of treatments was five (range, one to 15), delivered over 9 weeks. All patients received the full calculated dose at each treatment, but treatment interval varied. Most patients received three weekly treatments, followed by treatments every other week.

Therapeutic Results

Of the 30 patients, one achieved a complete response (3%) lasting 19+ months and four achieved partial responses (13%) lasting 4+, 5, 5, and 7+ months. Thus, the overall response rate for the entire group was 16% (table 1). There was no significant difference in response rate between patients who had received prior chemotherapy (13%) and those who had had no prior chemotherapy (21%).

There was no difference in the following prognostic variables between patients who responded and nonresponders: age, sex, primary site, and site of disease recurrence. No patients responded to treatment who had a Karnofsky status < 80 or who had a poorly differentiated or undifferentiated cancer.

The mean survival for the entire group was 4 months (1–23+). The mean survival was 8 months for the responders and 3 months for the nonresponders.

Toxicity

All patients were evaluable for toxicity (table 2). Ten patients developed neutropenia with a wbc count < 3000/mm^3, associated with one episode of aspiration pneumonia and death. Two patients had platelet counts < 100,000/mm^3, and one had local bleeding at the base of the tongue.

Seven patients developed mucositis; it was severe in three. Three patients had moderate to severe vomiting. Seven patients developed diarrhea, four requiring hospitalization. Three of the hospitalized patients had associated severe abdominal pain; one died with pseudomembranous colitis. One patient underwent laparotomy for suspected appendicitis; no lesion was found.

No patient developed a creatinine elevation > 1.5 mg/100 ml. One patient developed severe dermatitis. Seven patients had treatment discontinued because of toxicity: two had recurrent severe mucositis; one had severe dermatitis; three had severe diarrhea and abdominal pain; and one patient died of infection.

DISCUSSION

Chemotherapy has been only moderately effective in palliating recurrent squamous cancers of the head and neck (2,3). The most effective agents include methotrexate, cisplatin, bleomycin, and 5-FU (1,3,4), but response rates are low, and most responses are partial and of short duration. The goal of combination chemotherapy is to increase complete responses and survival without substantial toxicity. Multiple combinations have been developed but none achieve these goals better than single agents (8,11,12).

An innovative treatment of sequential methotrexate and 5-FU was developed by Pitman et al (9) based on studies by Cadman et al (10) showing synergistic tumor cell kill in vivo in mice with L1210 leukemia and in vitro. Since in vitro methotrexate concentrations > 10 μM were necessary to maximize the synergy, Pitman et al administered methotrexate at doses of 125–250 mg/m^2. This was followed 1 hour later by 5-FU (600 mg/m^2 iv). Leucovorin rescue was begun at 24 hours at a dose of 10 mg/m^2 every 6 hours for six doses. The regimen was given weekly to toxicity and then every 2 weeks. At

TABLE 1.—Results of treatment

	No. of patients	No. of— Complete responses	Partial responses	Overall responses
No prior treatment	14	0	3	3
Prior treatment	16	1	1	2
Total	30	1	4	5

TABLE 2.—Toxicity

	No. of patients
Total	30
Hematologic (cells/mm^3)	
Wbc count < 3000	10
Platelet count < 100,000	2
Gastrointestinal	
Mucositis*	
Grade 3	3
Vomiting†	
Grade 3	1
Grade 4	2
Diarrhea‡	
Grade 3	3
Grade 4	4

*Grade 3: confluent mucositis.

†Grade 3: 1–3 times daily. Grade 4: continuous, requires hospitalization.

‡Grade 3: ⩾ 4 movements/day. Grade 4: requires hospitalization.

the first report ten patients were evaluable and all achieved a response (> 50%); four had complete responses.

We have used this regimen on 30 patients, with only a 16% response (> 50%) rate; one complete response lasted 21+ months. There are several possible explanations for the wide difference in responses. The first relates to differences in patient population. Response rates to chemotherapy for head and neck cancer are higher in patients who have had no prior treatment (13). Almost one-half of the patients in the study of Pitman et al (9) had had no prior treatment, although the investigators did not find that this factor influenced response. All of our patients had had prior surgery and/or radiation. In addition, more than one-half of our patients had had prior chemotherapy. However, the response rate in those 14 patients who had had no prior chemotherapy was still only 21%. Another important prognostic variable is Karnofsky status (14); our mean was 80. However, the response rate for those patients with a Karnofsky status ⩾ 80 was still only 25%. Other factors in our patient population that may influence response, such as primary site (4), site of recurrence, and histologic differentiation (15), cannot be compared to those in the patients of Pitman et al (9).

A second reason for difference in response may relate to treatment. Our doses of chemotherapy were similar to those of the Yale group. The mean number of treatments per patient was five, compared to 7.7 in the patients of Pitman et al (9). Seven of our patients received only one or two treatments because of rapid progression or severe toxic effects. Pitman et al noted that all responses occurred by the sixth week. Our response rate was still only 29% in those 17 patients who received at least 6 weeks of treatment.

The toxic effects were similar, except that our patients had more severe neutropenia. This was significant in only one patient. The most toxic side effect in our series was severe diarrhea, which occurred in 23% of the patients. It was associated with acute abdominal pain in three patients after six, eight, and 12 treatments; there was one death. Bertino (personal communication) has suggested that this toxicity may be reduced by three weekly treatments, followed by treatments every 2 weeks.

In summary, the combination of methotrexate plus 5-FU at our institution produced durable responses (4+ to 19+ months), but the number of responses was low. We cannot explain why this is so different from the Yale series. We cannot recommend this regimen for general use until other groups confirm the initially reported high response rate or it is shown to be superior to methotrexate alone in a randomized trial.

REFERENCES

1. CARTER SK. The chemotherapy of head and neck cancer. Semin Oncol 4:413–424, 1977.
2. GLICK JH, and TAYLOR SG. Integration of chemotherapy into a combined modality treatment plan for head and neck cancer: a review. Int J Radiat Oncol Biol Phys 7:229–242, 1981.
3. MEAD G, and JACOBS C. The changing role of chemotherapy in the treatment of head and neck cancer. Am J Med. In press.
4. BERTINO JR, BOSTON B, and CAPIZZI RL. The role of chemotherapy in the management of cancer of the head and neck: a review. Cancer 36:752–758, 1975.
5. LIVINGSTON RB, EINHORN LH, BURGESS MA, ET AL. Sequential combination chemotherapy for advanced, recurrent, squamous carcinoma of the head and neck. Cancer Treat Rep 60:103–105, 1976.
6. CARADONNA R, PALADINE W, RUCKDESCHEL JC, ET AL. Methotrexate, bleomycin, and high-dose *cis*-dichlorodiammineplatinum(II) in the treatment of advanced epidermoid carcinoma of the head and neck. Cancer Treat Rep 63:489–491, 1979.
7. WITTES RE, SPIRO RH, SHAH J, ET AL. Chemotherapy of head and neck cancer; combination treatment with cyclophosphamide, adriamycin, methotrexate, and bleomycin. Med Pediatr Oncol 3:301–309, 1977.
8. KAPLAN BH, SCHOENFELD D, and VOGL SE. Treatment of recurrent or metastatic squamous cancer of the head and neck with methotrexate (M), plus *Corynebacterium parvum* or M plus bleomycin (B) plus diamminedichloroplatinum (D)—a prospective randomized trial of the Eastern Cooperative Oncology Group. Proc Am Assoc Cancer Res and ASCO 22:532, 1981.
9. PITMAN SW, KOWAL CD, PAPAC RI, ET AL. Sequential methotrexate-5-fluorouracil: a highly active drug combination in advanced squamous cell carcinoma of the head and neck. Proc Am Assoc Cancer Res and ASCO 21:473, 1980.
10. CADMAN E, HEIMER R, and DAVIS L. Enhanced 5-fluorouracil nucleotide formation after methotrexate administration: explanation for drug synergism. Science 205:1135–1137, 1979.
11. BAKER L, and AL-SARRAF M. A comparative trial of cisplatinum (C), Oncovin (O), and bleomycin (B) versus methotrexate (M) in patients with advanced epidermoid carcinoma of the head and neck. Proc Am Assoc Cancer Res and ASCO 20:202, 1979.
12. DAVIS S, and KESSLER W. Randomized comparison of *cis*-diamminedichloroplatinum versus *cis*-diamminedichloroplatinum, methotrexate, and bleomycin in recurrent squamous cell carcinoma of the head and neck. Cancer Chemother Pharmacol 3:57–59, 1979.

13. Elias GE, Chretien PB, Monnard E, et al. Chemotherapy prior to local therapy in advanced squamous cell carcinoma of the head and neck: preliminary assessment of an intensive drug regimen. Cancer 43:1025–1031, 1979.
14. Amer MH, Al-Sarraf M, and Vaitkevicius VK. Factors that affect response to chemotherapy and survival of patients with advanced head and neck cancer. Cancer 43:2202–2206, 1979.
15. Soper WT, and Gott AB. Clinical brochure. New Drug Seminar on Bleomycin. Bethesda, MD, National Cancer Institute, 1974.

Phase II Trial of Mitoxantrone[1]

Kenneth C. Anderson,* Gary I. Cohen, and Marc B. Garnick[2]

Systematic substitutions on the basic anthraquinone nucleus have produced mitoxantrone (DHAD) (1), a compound with significant antitumor activity in animal tumor systems (2), which produces DNA single-strand breaks and high relative cytotoxicity as noted with doxorubicin (ADR) (3). Moreover, DHAD was shown to be less cardiotoxic in the rat model (4), in beagles, and in cynomolgus monkeys (5). Hepatobiliary excretion constitutes the main route of drug elimination, with a half-life of 112.8 minutes (6). Phase I–II studies (7–14) indicated that the dose-limiting toxicity was leukopenia. A phase II study of DHAD was undertaken using a starting dose of 12 mg/m^2 administered iv over 1 hour every 21 days.

MATERIALS AND METHODS

All patients had histologic confirmation of refractory cancer, were ambulatory, and had normal hematologic, renal, cardiac, and hepatic functions. Measurable disease and a life expectancy of at least 2 months were required. Prior treatment with ADR ($\leq$ 450 mg/m^2) did not exclude patients from this study. A starting iv dose of 12 mg/m^2 was administered by dilution of DHAD in 200 ml of saline infused over 60 minutes every 21 days, with the dose modifications shown in table 1.

Evaluation for response and toxicity usually required multiple DHAD treatments and observation for at least 21 days thereafter.

TABLE 1.—Criteria for dose modification

Granulocyte count nadir (cells/mm^3)	Platelet count nadir (cells/mm^3)	Total bilirubin (mg/dl)	Dose adjustment
> 2000	> 150,000	< 1.5	Increase by 1 mg/m^2
1000–2000	75,000–150,000	< 1.5	Maintain same dose
< 1000	< 75,000	1.5–3.0	Decrease by 1 mg/m^2

RESULTS

Seventy-seven patients (190 courses) were evaluable for response and toxicity (table 2). Ninety percent of the patients had received prior therapy: combination chemotherapy (43%), radiation therapy (7%), or both (40%). Twenty-seven patients had had prior ADR exposure; ADR had been discontinued in 23 (85%) because of progressive disease, while four (15%) had previously responded to ADR either as a single agent or in combination programs. Sixty of the 77 patients received more than one course of DHAD therapy; dose escalation was achieved in 22 patients. Of 13 patients who developed leukopenia requiring dose reduction, seven had evidence of cumulative myelosuppression.

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Entered	86*
Evaluable	77†
Male/female	47/30
Median age in yrs (range): 57 (22–78)	
Prior treatment	
Chemotherapy	33
ADR	27‡
Radiotherapy	5
Chemotherapy and radiotherapy	31
No prior therapy	8

*9 patients inevaluable due to inappropriate (low) dose modifications.
†Total No. of courses = 190.
‡Median dose = 400 mg/m^2; range, 60–500 mg/m^2.

Toxicity (Table 3)

Thirteen of 190 courses (7%) were associated with severe myelosuppression (granulocyte count nadir < 1000 mm^3) at a median of 11 days following drug

[1]Received Apr 28, 1982; revised July 30, 1982; accepted Aug 16, 1982.
[2]Division of Medicine, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA.

*Reprint requests to: Kenneth C. Anderson, MD, Division of Medicine, Sidney Farber Cancer Institute, Harvard Medical School, 44 Binney St, Boston, MA 02115.

TABLE 3.—Toxic effects

Toxic effect	No. of patients	% of patients (n = 77)	% of courses (n = 190)
Nausea/vomiting	2	3	2
Phlebitis	1	1	1
Rash	1	1	1
Granulocyte count nadir (cells/mm^3)*			
< 1000	13	17	7
1000–1999	30	39	17
2000–3000	22	29	29
Platelet count nadir ≤ 75,000 cells/mm^3†	4	5	2
Clinical hemorrhage	0	0	0
Sepsis/death‡	2	3	1
Cardiotoxicity	0	0	0

*65 of 77 patients (84%) ultimately developed granulocyte count nadirs < 3000/mm^3.
†No clinical hemorrhage.
‡71-year-old female who had received prior radiotherapy and methyl-GAG and vinblastine chemotherapy for metastatic renal cancer had a granulocyte count of 400/mm^3 3 days after 3rd 12-mg/m^2 dose of DHAD and died of staphylococcal sepsis in spite of antibiotic therapy. 46-year-old male with colorectal cancer metastatic to lung and liver, who had received prior 5-FU therapy, received 12- and 13-mg/m^2 doses of DHAD and had a granulocyte count nadir > 2000/mm^3. He developed a granulocyte count of 1000/mm^3 4 days after a 14-mg/m^2 dose of DHAD and died of gram-negative sepsis in spite of antibiotic therapy.

administration. In 65 of 77 patients (84%), 102 of 190 courses (53%) resulted in myelosuppression (granulocyte count nadir < 3000/mm^3). Thrombocytopenia (platelet count nadir < 75,000/mm^3) occurred in 2% of the courses, but platelet transfusions were not required. Nausea and vomiting were infrequent. Phlebitis at the site of drug infusion occurred only once and resolved promptly with hydrocortisone therapy. One patient developed a diffuse pruritic erythematous maculopapular eruption, which resolved promptly on discontinuation of the drug. None of the patients had clinical or electrocardiographic evidence of cardiac dysfunction, including 27 patients who had received prior ADR chemotherapy.

Response to DHAD Therapy (Table 4)

No patient achieved a complete response. Well-documented partial response was achieved by five patients with the following tumors (table 5): lung adenocarcinoma (two patients), breast carcinoma (one), colorectal carcinoma (one), and melanoma (one). Four patients who had colorectal carcinoma and rapidly progressing liver metastases before DHAD treatment had either disease stabilization (cessation of progression) or < 50% regression of measurable lesions for 1–2.5 months after DHAD treatment. Sixty-eight of the 77 patients (88%) had clear-cut disease progression occurring after two or three courses of DHAD. This included three of

TABLE 4.—Therapeutic response to DHAD

Diagnosis	No. of patients (%)				
	Total	Partial response	Stable disease	Progressive disease	Prior ADR therapy
Non-oat cell lung cancer	17	2* (12)	0 (0)	15 (88)	13 (76)
Renal cancer	10	0 (0)	0 (0)	10 (100)	1 (90)
Breast cancer	4	1* (25)	0 (0)	3 (75)	4 (100)
Melanoma	7	1 (14)	0 (0)	6 (86)	0 (0)
Colorectal cancer	29	1† (3)	4 (14)	24 (86)	0 (0)
Sarcoma	4	0 (0)	0 (0)	4 (100)	4 (100)
Other	6	0 (0)	0 (0)	6 (100)	5 (83)
Total	77	5 (7)	4 (5)	68 (88)	27 (35)

*1 patient with prior ADR therapy.
†1 patient with prior 5-FU/PALA therapy.

TABLE 5.—Partial responders to DHAD

Age	Sex	Diagnosis	Prior therapy*	Total dose of DHAD (mg/m²)	Response	Response duration (mos)
61	M	Non-oat cell lung cancer	None	60	> 50% ↓ lung mass; CEA, 15 → 3 ng/ml	4.0
72	M	Non-oat cell lung cancer	C, ADR, P, XRT	36	> 50% ↓ hilar mass; CEA, 270 → 130 ng/ml	4.5
54	F	Breast cancer	BS + O, C, M, 5-FU, ADR	96	> 50% ↓ bony metastases; CEA, 170 → 85 ng/ml	2.0
75	M	Colorectal cancer	5-FU, PALA	73	> 50% ↓ hepatic metastases	4.0
72	M	Melanoma	None	34	> 50% ↓ pulmonary nodules	1.5

*C = cyclophosphamide; P = cisplatin; XRT = radiation therapy; BS + O = bilateral salpingo-oophorectomy; and M = methotrexate.

four patients with breast cancer who had previously responded to ADR therapy.

DISCUSSION

The data of this phase II study in selected solid neoplasms suggest that DHAD has activity against non-oat cell lung cancer but not colorectal cancer. Inadequate numbers of patients with melanoma and breast cancer make any conclusions premature. An overall objective partial response was observed in five of 77 evaluable patients (7%). The observed partial response rate in patients with non-oat cell lung carcinoma is 12% (two of 17 patients), with a $\geq$ 90% probability that the observed response will be 0–20%. One of these patients and the responding patient with breast carcinoma both had disease progression after receiving 450 mg/m² of ADR. The response rate of 3% in 29 patients with colorectal carcinoma assures with a $\geq$ 90% probability that the true response rate is $\leq$ 5%.

The dose-limiting toxic effect in all phase I–II studies of patients treated with DHAD has been leukopenia (7–14), as was true in our study. Although cardiotoxicity after DHAD treatment has been documented in nine patients previously treated with ADR (L. Lev, personal communication, Oct 1981), 27 patients in our study who had been previously treated with ADR manifested no cardiotoxicity as monitored by clinical assessment and electrocardiography.

In summary, this study confirms the mild toxicity of DHAD administered in a 12-mg/m² iv bolus dose every 3 weeks. Partial responses were documented in non-oat cell lung cancer, breast cancer, colorectal cancer, and melanoma. Prior therapy with ADR did not predispose to resistance to DHAD. Although no cardiotoxicity was observed in this study, it is premature to definitively conclude that prior ADR therapy does not predispose to DHAD-related cardiotoxicity.

REFERENCES

1. JOHNSON RK, ZEE-CHENG RKY, LEE WW, ET AL. Experimental antitumor activity of aminoanthraquinones. Cancer Treat Rep 63:425–439, 1979.
2. WALLACE RE, MURDOCK KC, ET AL. Activity of a novel anthracenedione, 1,4-dihydroxy-5,8-bis[(2-((2-hydroxyethyl)amino) ethyl)amino]-9,10-anthracenedione dihydrochloride, against experimental tumors in mice. Cancer Res 39:1570–1574, 1979.
3. COHEN LF, GLAUBIGER DL, KARN HE, ET AL. Protein associated DNA single strand breaks and cytotoxicity of dihydroxyanthracenedione (DHAD), NSC-301739, in mouse L1210 leukemia cells. Proc Am Assoc Cancer Res and ASCO 21:277, 1980.
4. CHENG CC, ZBINDEN G, and ZEE-CHENG RKY. Comparison of antineoplastic activity of aminoethyl-aminoanthraquinones and anthracycline antibiotics. J Pharm Sci 68:393, 1979.
5. 1,4-Dihydroxy-5,8-bis[(2-((2-hydroxyethyl) amino) ethyl)]-9,10-anthracenedione (CL 232, 314 NSC 301739). Investigator's brochure available through the National Cancer Institute from American Cyanamid Co, Medical Research Division, Pearl River, NY.
6. NIEDHART J, STAUBUS A, YOUNG D, ET AL. Pharmacokinetic studies of dihydroxyanthracenedione (DHAD, NSC 301739) with clinical correlations. Proc Am Assoc Cancer Res and ASCO 22:363, 1981.
7. MESHAD MW, GARNICK MB, PEGG WJ, ET AL. Phase I trial of 1,4-dihydroxy-5,8-bis[(2-((2-hydroxyethyl)amino)ethyl)]-9,10-anthracenedione by 24 hour continuous infusion. In press.
8. VON HOFF DD, POLLARD E, KUHN J, ET AL. Phase I clinical investigation of DHAD, a new anthracenedione. Cancer Res 40:1516, 1980.
9. YAP HY, BLUMENSCHEIN GR, SCHELL FC, ET AL. Dihydroxyanthracenedione: a promising new drug in the treatment of metastatic breast cancer. Ann Intern Med 95(6):694, 1981.
10. WYNERT W, HARVEY H, LIPTON A, ET AL. Phase I study of dihydroxyanthracenedione (mitoxantrone). Proc Am Assoc Cancer Res and ASCO 22:352, 1981.
11. VALDIVIESO M, BEDIKIAN AY, BURGESS MA, ET AL. Phase I clinical study of dihydroxyanthracenedione administered on a 5-day iv schedule. Cancer Treat Rep 65:841–844, 1981.
12. VAN ECHO DA, WHITACRE MY, AISNER J, ET AL. Phase I trial of dihydroxyanthracenedione. Cancer Treat Rep 65:831–834, 1981.
13. ESTEY EH, KEATING MJ, MCCREDIE KB, ET AL. Phase I–II study of dihydroxyanthracenedione (DHAD) in acute leukemia (AL). Proc Am Assoc Cancer Res and ASCO 22:490, 1981.
14. UNGERLEIDER RS, COHEN LF, GLAUBIGER DL, ET AL. Dihydroxyanthracenedione (NSC 301739, mitoxantrone): phase I evaluation in children. Proc Am Assoc Cancer Res and ASCO 22:392, 1981.

Cisplatin Urinary Pharmacokinetics and Nephrotoxicity: A Common Circadian Mechanism[1,2]

Francis Levi, William J. M. Hrushesky,* Richard F. Borch, Michael E. Pleasants, B. J. Kennedy, and Franz Halberg[3]

Renal toxicity and urinary pharmacokinetics following a nonlethal dose (5 mg/kg) of cisplatin were investigated in female F344 (Fischer) rats that were on a standardized light-dark schedule, with water freely available. Circadian timing of drug administration had a major effect on the BUN increase ($P < 0.01$), the urine volume increase ($P < 0.025$), and the urinary concentration of cisplatin following drug administration ($P < 0.01$). Renal toxicity was positively correlated with the peak urinary concentration of cisplatin and with the area under the curve of urinary concentration over the first 20 hours after drug administration ($P < 0.01$). Drug administration near the normal circadian maximum of urinary volume prior to treatment resulted in the least renal toxicity, lowest peak, and smallest area of urinary concentration of cisplatin. [Cancer Treat Rep 66:1933–1938, 1982]

Cisplatin is an important anticancer drug that has exhibited activity against a wide variety of human malignancies (6,25). Its dose-limiting side effect is irreversible nephrotoxicity (4,22), and the microscopic finding of tubular necrosis is common following cisplatin administration (8,18). It has been suggested that the urinary concentration of cisplatin may determine the amount of cisplatin-induced renal damage (3,9). This hypothesis is supported by the fact that aggressive hydration protocols reduce the nephrotoxicity of this drug (2,16,24).

Renal function is very rhythmic on the circadian time scale (5,15,26–28). The total extent of predictable circadian variation of most urinary variables investigated in rodents and human beings ranges between 100% and 200% of the 24-hour mean (11), and these circadian rhythms in renal function persist under various environmental conditions.

In previous murine work (10,17,20), we have demonstrated that a large-amplitude circadian rhythm characterizes renal tolerance for a high dose of cisplatin (11 mg/kg). Optimal renal tolerance for this drug occurs very near the time of day associated with the normal circadian maximum in urinary volume. The protective effect of concurrent hydration is largest when both cisplatin and hydration are given at this optimal time. Conversely, when both agents are administered near the time of day associated with the circadian minimum in urinary volume, little, if any, renal protection results from hydration. The studies reported here investigate whether a circadian rhythm in renal tolerance for a nonlethal dose of cisplatin exists in two different strains of rats (Fischer and Louvain) and whether the urinary pharmacokinetics of this drug differ significantly as a function of circadian timing of cisplatin administration.

MATERIALS AND METHODS

Study Designs

Studies 1 and 2.—Study 1 (May 28, 1978) and study 2 (Sept 21, 1978) were performed with a total of 23 singly housed female F344 (Fischer) rats, 2–6 months of age and weighing 90–180 g. These animals were standardized to a schedule of 8 hours of light alternating

[1]Received Nov 2, 1981; revised July 13, 1982; accepted Aug 9, 1982.

[2]Supported by Public Health Service grants GM-13981 from the National Institute of General Medical Sciences, CA-31635 and CA-14445 from the National Cancer Institute, AG-00158 from the National Institute on Aging (National Institutes of Health), and OH-00631 from the National Institute of Occupational Safety and Health, Department of Health and Human Services; and by grants from the Masonic Memorial Hospital Fund, Inc; the Minnesota Medical Foundation; the American Cancer Society; and the St. Louis Park Medical Center Research Foundation (#298-81). Dr. Hrushesky is an American Cancer Society junior faculty fellow (grant 509A).

[3]Section of Medical Oncology, Department of Medicine, (*F. Levi, W. J. M. Hrushesky,* and *B. J. Kennedy*), Department of Chemistry (*R. F. Borch* and *M. E. Pleasants*), and Chronobiology Laboratories (*F. Halberg*), University of Minnesota Medical School, Minneapolis.

**Reprint requests to:* Dr. William Hrushesky, Section of Medical Oncology, Department of Medicine, PO Box 286, University of Minnesota Hospitals, Minneapolis, MN 55455.

with 16 hours of darkness for 3 weeks prior to and throughout the study. In order that food not contaminate urine specimens, food availability was progressively restricted to the first 2 hours of darkness. Fourteen rats were kept in plastic cages, and nine rats were housed in metabolic cages. For 10 days before and 6 days after drug administration, urine samples were obtained every 4 hours (using an automatic urine collector) from the animals kept in metabolic cages. Two times were selected for cisplatin injection: that of the maximum (acrophase) and that of the minimum (bathyphase) of the mean circadian rhythm in urine volume assessed before drug administration. The body weight of each rat was measured daily at 8 hours after light onset (8 HALO) during the 6-day span following drug injection.

Isolation of animals was necessary to obtain accurate measurements of urine volume from individual rats. The effect of isolation on rats must be considered, but this variable was kept constant across all groups.

Study 3.—Twenty-four female LOU (Louvain) rats, 2–6 months of age and weighing 100–200 g, were singly housed in plastic cages and standardized to a schedule of 12 hours of light alternating with 12 hours of darkness for 5 weeks before and throughout the study. On Nov 1, 1980, the rats were randomized to receive a single ip injection of cisplatin (5 mg/kg) or normal saline (controls) at 7 HALO or 19 HALO. These two circadian stages, respectively, correspond to the mean nadir and mean maximum of the circadian rhythm in urine volume of rats fed ad libitum (14). At 7 HALO 2.5 or 3 days later, the rats were weighed and rectal temperature was measured with a thermistor probe. The rats were then killed by decapitation and blood was collected. Serum was analyzed for BUN content. Both kidneys were removed, fixed in Formalin, and stained with hematoxylin and eosin.

Drug Preparation

Cisplatin was supplied by the Investigational Drug Branch, National Cancer Institute, in vials containing 10 mg of cisplatin plus mannitol and sodium chloride. The final solution used for injection contained 1 mg of cisplatin, 10 mg of mannitol, and 9 mg of sodium chloride per milliliter. Fresh drug was prepared for each injection.

Cisplatin Determination

Exactly 3.0 ml of urine was adjusted to pH 8 with 5% aqueous sodium carbonate solution, and 100 µl of 0.5 M aqueous diethylammonium diethyldithiocarbamate was added. The resulting solution was heated on the steam bath for 10 minutes and allowed to cool to room temperature, and exactly 3.0 ml of chloroform was added. This mixture was vortexed for 5 minutes, aqueous NaSH (1 ml, 0.2 M) was added, and vortexing was continued for an additional 2–3 minutes. The chloroform layer was removed and analyzed for platinum concentration by high-performance liquid chromatography as described previously (1). Each determination was carried out in duplicate on freshly thawed urine.

Previous studies have shown that most of the platinum excreted in the urine within 4 hours of iv administration is in the form of unchanged cisplatin (3). The amount of cisplatin in 1 ml of urine can be calculated by multiplying the concentration of platinum by the molecular weight of cisplatin divided by that of platinum, a factor of 1.54.

Blood Urea Nitrogen

BUN was determined in serum by the urease method.

Histopathologic Assessment

Hematoxylin-eosin-stained slides of a slice of one kidney per rat were examined with light microscopy by an observer who did not know the treatment group of the donor rat. A semiquantitative assessment of the number of naked nuclei present within the tubular lumen was tabulated as a measure of the severity of cisplatin-induced renal damage. A scoring system was devised which ranked the amount of tubular damage from 0 (very few nuclei within the tubular lumina) to 5 (many nuclei within the tubular lumina).

Statistical Methods

Student's t tests were used to compare treatment groups. In addition, the single and population mean cosinor methods (12,13,23) were employed to determine and quantify the characteristics of any observed circadian rhythm. A P value from an F test of the zero-amplitude hypothesis is obtained. The fit of a cosine function to the data yields three rhythm characteristics with their respective 95% confidence limits. These are the mesor (rhythm-adjusted mean), the double amplitude (total extent of *predictable* variation accounted for by the cosine curve best fitting the data), and the acrophase (timing of the maximum of this best-fitting cosine curve). A fourth value, the percent rhythm, is a measure of the overall variability of the data which is attributable to the rhythm present.

RESULTS

Body Weight Loss

Studies 1 and 2.—Rats kept in metabolic cages lost 16.4% ± 2.0% (mean ± SE) of their body weight within the 6-day span following cisplatin administration, as

compared to 10.1% ± 0.6% for those animals housed in plastic cages ($t = 7.4$, $P < 0.001$). A difference between the two groups receiving cisplatin at different circadian stages was demonstrable (table 1), with statistical significance for the groups kept in plastic cages but not for the groups housed in metabolic cages.

Study 3.—All rats were weighed at 7 HALO approximately 3 days after cisplatin administration. The mean (± SE) body weight of control rats was 159.6 ± 2.7 g; rats receiving cisplatin 3 days earlier at 7 HALO weighed 151.3 ± 3.3 g, while those receiving cisplatin 3 days earlier at 19 HALO weighed 160.5 ± 4.0 g ($F = 3.2$, $P = 0.09$).

TABLE 1.—Body weight loss of female F344 rats (kept in plastic cages) on Day 6 after a single ip injection of 5 mg/kg of cisplatin

	% body weight loss*	
Injection time	Mean ± SE	Significance
Daily urine volume maximum	9.6 ± 0.4	$t = 2.15$
Daily urine volume minimum	12.4 ± 1.3	$P < 0.05$

*Body weight of each rat measured at 8 HALO and expressed as a % of its preinjection value.

Urine Volume Increase

Urine production before and after cisplatin administration was evaluated in each individual rat. Cisplatin administration resulted in an overall increase in the 24-hour mean of urine volume. This increase was maximal near the fifth day following drug administration. The increase in urine excretion over the first 6 days following cisplatin administration depended markedly on the circadian timing of drug injection ($P = 0.025$) (table 2).

TABLE 2.—Cisplatin-induced polyuria in female F344 rats secondary to ip injection of 5 mg/kg of cisplatin*

	Integrated relative increase in urine volume	
Injection time	% × days (mean ± SE)	Significance
Daily urine volume maximum	593 ± 138	$t = 2.9$
Daily urine volume minimum	1396 ± 263	$P = 0.025$

*Area of relative response over 6 days following ip drug administration at circadian acrophase or bathyphase of urine volume.

Blood Urea Nitrogen

When cisplatin was given at 19 HALO, the BUN was only slightly elevated (25 ± 1 mg/dl) when compared with that of controls (17 ± 1 mg/dl), whereas the administration of cisplatin at 7 HALO resulted in a threefold rise in BUN (53 ± 5 mg/dl) (fig 1).

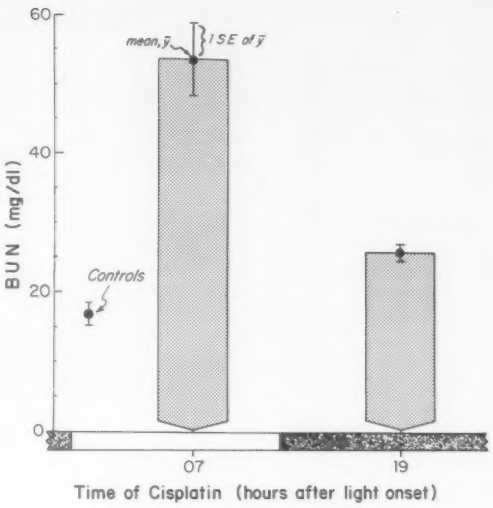


FIGURE 1.—BUN ~ 3 days after administration of single ip dose of cisplatin to female LOU rats on standardized schedules. Drug given at 7 or 19 HALO. Blood obtained at 7 HALO ~ 3 days later. Role of circadian time: $t = 4.4$, $DF = 14$, $P < 0.001$. Cisplatin at 19 HALO vs controls: $t = 5.0$, $DF = 14$, $P < 0.001$.

Histologic Changes

Histopathologic lesions predominated in the proximal tubule. They consisted of hydropic and necrotic changes. Proximal tubular cells appeared swollen. Caryorrhexis and pyknotic nuclei were noted. The scoring system revealed a mean ± SE of 0 ± 0 for controls, 2.9 ± 0.4 for rats receiving cisplatin at 7 HALO, and 1.7 ± 0.7 for rats receiving cisplatin at 19 HALO ($t = 1.8$, $0.10 < P < 0.20$). The correlation between histopathologic score and BUN was statistically significant ($r = 0.70$, $P < 0.05$).

Cisplatin Urinary Excretion

Figure 2 summarizes the urinary excretion of cisplatin. Irrespective of injection time, most cisplatin recovered in the urine was found in the first 8 hours following drug injection. Rats injected with cisplatin near the circadian maximum of urine volume excreted more of the drug in the Hour 4 urine sample and less drug in the Hour 8 sample than rats receiving cisplatin near the circadian minimum in urine volume.

The total urinary excretion of cisplatin (expressed as percent of the injected dose) in the first 20 hours following drug injection was 41.1 ± 3.6 in rats injected near the time of the daily maximum of urine volume and 33.2 ± 0.8 in rats injected near the daily nadir of urine volume ($t = 2.0$, $0.05 < P < 0.10$).

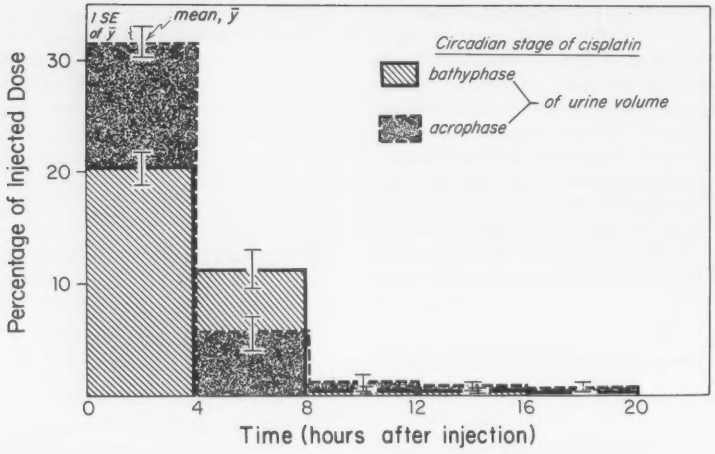


FIGURE 2.—Cisplatin urinary excretion expressed as % of injected dose recovered every 4 hrs over 20 hrs following ip administration of 5 mg/kg of the drug to female F344 rats on standardized schedules. Cisplatin was given at maximum or minimum of mean circadian rhythm in pretreatment urine volume.

Cisplatin Urinary Concentration

The urinary concentration of cisplatin in both studies is shown in figure 3. In the Hour 4 urine sample obtained from rats receiving cisplatin near the circadian maximum of urine volume, cisplatin concentration was one-half of that observed after cisplatin administration near the circadian minimum of urine volume ($P < 0.01$). This difference was even larger in the Hour 8 urine sample.

Table 3 demonstrates the effect of cisplatin timing on the area under the curve of urinary drug concentration during the first 20 hours following drug administration. Cisplatin urinary concentration may reflect the drug concentration to which proximal tubular cells are exposed. If so, during the 20-hour span following cisplatin injection, the tubular cells of rats receiving cisplatin near the time of day associated with the daily maximal urine volume were exposed to about half the cisplatin

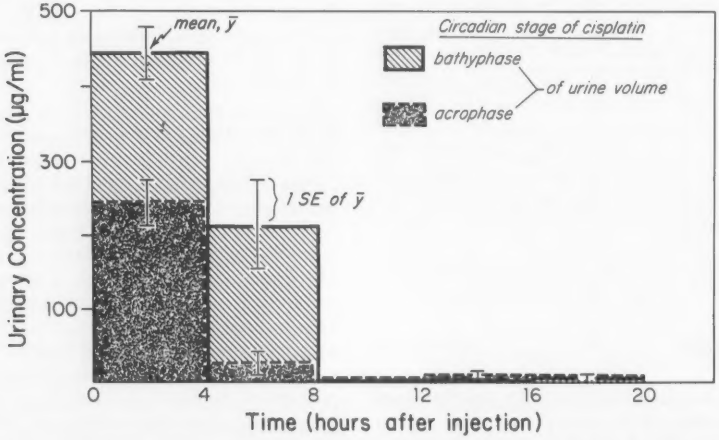


FIGURE 3.—Cisplatin urinary concentration of 4-hr urine specimens collected over 20 hrs following ip administration of 5 mg/kg of the drug to female F344 rats on standardized schedules. Cisplatin was given at maximum or minimum of mean circadian rhythm in pretreatment urine volume.

concentration as were the tubular cells of rats injected at the time of day associated with the daily minimum.

TABLE 3.—Total cisplatin urinary concentration over the 20-hr span (C × T) following ip administration of 5 mg/kg of the drug to F344 rats

	Total cisplatin concentration	
Injection time	C × T* (mean ± SE)	Significance
Daily urine volume maximum	1257 ± 197	$t = 3.7$
Daily urine volume minimum	2470 ± 278	$P = 0.01$

*$\mu g \times hr \times ml^{-1}$.

Urinary Pharmacokinetics and Nephrotoxicity of Cisplatin

A statistically significant correlation was demonstrated between the areas for cisplatin urinary concentration × time and for urinary volume response × time, evaluated over the first 6 days following drug injection (fig 4). A statistically significant correlation was also found between this apparent loss of urinary concentrating ability and the peak of cisplatin urinary concentration ($r = 0.79$, $N = 9$, $P < 0.025$).

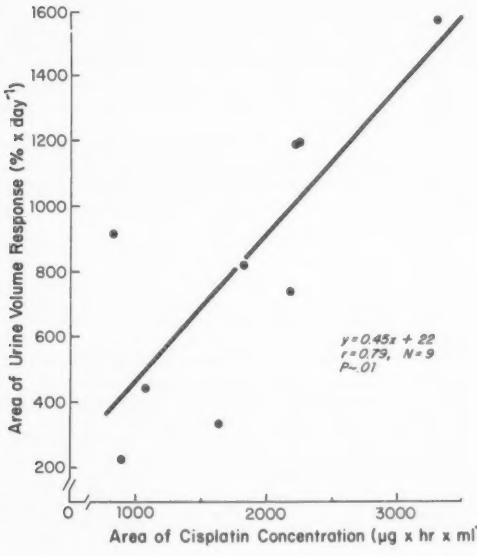


FIGURE 4.—Correlation between cisplatin nephrotoxicity gauged by urine volume response and urinary drug concentration in 9 female F344 rats singly housed in metabolic cages and standardized to light: darkness schedules of 8:16 hrs, with food availability restricted to first 2 hrs of darkness. Areas of cisplatin concentration over 20-hr span after cisplatin. Areas of relative urine volume response over 6-day span following drug injection.

DISCUSSION

Urinary volume increase as the result of a presumed loss of urinary concentrating ability has been demonstrated to be a sensitive index of cisplatin-induced renal damage in the rat (7,19). In these studies, we have found that this index of cisplatin nephrotoxicity depends largely on the circadian timing of a nonlethal dose of this drug. We also demonstrated that cisplatin-induced renal toxicity is circadian stage-dependent in two strains of rats under different standardized lighting and feeding regimens.

In study 3, the interval between cisplatin administration and blood sampling for BUN determination differed by 12 hours between animals injected at 7 or 19 HALO, because rats receiving cisplatin at circadian stages 12 hours apart were all sampled at the same time. Our choice of sampling all rats at the same circadian stage (7 HALO) as opposed to sampling all rats after the same span following cisplatin was essential to avoid the confounding effect of the normally occurring circadian rhythm in BUN. In both transverse and longitudinal study designs, we have shown that within a 24-hour range, the circadian stage of sampling is critical. This has proven to be true for assessing cisplatin renal toxicity by either systemic endpoints (body weight or temperature) or by the kidney-specific indicators of urine volume, BUN, or urinary *N*-acetylglucosaminidase activity (19,21).

According to Guarino et al (9), direct tubular exposure to a concentration of 200 μg/ml of cisplatin for a few hours is necessary for tubular damage to occur. Our data demonstrate that a concentration of this magnitude persists 6 ± 2 hours in the excreted urine if cisplatin is given at the time of day associated with the circadian minimum of urine volume, whereas it only lasts 2 ± 2 hours if the drug is given near the circadian maximum of urine volume.

The pharmacokinetics of cisplatin urinary concentration in the rat differ tremendously as a function of drug timing. The statistically significant correlation between these pharmacokinetic parameters and subsequent week-long loss of urinary concentrating ability supports the role of the urinary elimination of cisplatin in its nephrotoxicity. Although some authors infer a simple direct relationship between urinary peak concentration of cisplatin and the renal toxicity of this drug (2,9), our work suggests a similar but more complex hypothesis.

We have demonstrated elsewhere that cisplatin nephrotoxicity is correlated with the urinary activity of β-*N*-acetylglucosaminidase (NAG)—a lysosomal enzyme released into the urine from the brush border of proximal tubular cells. We have also reported that platinum uptake by many tissues is positively correlated with NAG tissue activity (20).

In the unperturbed state, a statistically highly sig-

nificant circadian rhythm in urinary NAG is present. The circadian amplitude of this normal urinary rhythm approximates 100% of the 24-hour mean. Urinary NAG activity, as well as other urinary enzymatic activities specific to the proximal tubule, may reflect a highly specific index of the ability of platinum to enter the proximal tubular cell. Most likely, renal and subsequent urinary NAG activities reflect rhythmic renal metabolic activity. Proximal tubular reabsorption is maximal when the activities of NAG and other enzymes are highest. Cisplatin uptake by these proximal tubular cells and the resulting cellular damage may depend on the *interaction* of (*a*) the circadian stage of metabolic activity of the renal tubular cells as reflected by their NAG content at the time of cisplatin exposure and (*b*) the urinary concentration of cisplatin in the proximal tubule. The concentration of cisplatin in the excreted urine depends on both of these parameters.

This hypothesis can help explain why the degree of renal protection which results from the concurrent administration of identical hydration regimens is either trivial or very large, depending on whether cisplatin is given at a circadian stage of maximal or minimal metabolic activity of the proximal tubular cells, respectively (17,20).

REFERENCES

1. Borch RF, Markovitz JH, and Pleasants ME. A new method for the HPLC analysis of Pt (II) in urine. Anal Lett 12:917–926, 1979.
2. Cvitkovic E, Spaulding J, Bethune V, et al. Improvement of cisdichlorodiammineplatinum (NSC 119875): therapeutic index in an animal model. Cancer 39:1357–1361, 1977.
3. DeConti RC, Toftness BR, Lange RC, et al. Clinical and pharmacological studies with *cis*-diamminedichloroplatinum (II). Cancer Res 33:1310–1315, 1973.
4. Dentino M, Luft FC, Yum MN, et al. Long-term effect of *cis*-diamminedichloroplatinum (CDDP) on renal function and structure in man. Cancer 41:1274–1281, 1978.
5. Doe RP. A study of the circadian variation in adrenal function and related rhythms. PhD thesis. Minneapolis, University of Minnesota, 1966.
6. Einhorn LH, and Donohue J. *cis*-Diamminedichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293–298, 1977.
7. Goldstein RS, Noordevier B, Bond JT, et al. Manifestation of *cis*-diamminedichloroplatinum (II) nephrotoxicity. Clin Res 28:742A, 1980.
8. Gonzalez-Vitale JC, Hayes DM, Cvitkovic E, et al. The renal pathology in clinical trials of cisplatinum (II) diamminedichloride. Cancer 39:1362–1371, 1977.
9. Guarino AM, Miller DS, Arnold ST, et al. Platinate toxicity: past, present, and prospects. Cancer Treat Rep 63:1475–1483, 1979.
10. Halberg E, Halberg F, Vener KJ, et al. Twenty-four hour synchronized chronotolerance of *cis*-diamminedichloroplatinum (II) by rats on 8-h and 12-h photofraction gauged by acrophase and paraphase of rectal temperature. *In* Chronopharmacology (Reinberg A, and Halberg F, eds). Oxford/New York, Pergamon Press, 1979, p 377.
11. Halberg F. Chronobiology. Ann Rev Phys 31:675–725, 1969.
12. Halberg F, Carandente F, Cornelissen G, et al. Glossary of chronobiology. Chronobiologia 1:189, 1979.
13. Halberg F, Johnson EA, Nelson W, et al. Autorhythmometry procedures for physiologic self-measurements and their analysis. Physiol Teach 1:1–11, 1972.
14. Halberg F, Nelson W, Runge W, et al. Plans for orbital study of rat biorhythms. Results of interest beyond the biosatellite program. Space Life Sci 2:437–471, 1971.
15. Halberg F, Reinhardt J, Bartter FC, et al. Agreement in endpoints from circadian rhythmometry on healthy human beings living on different continents. Experientia 25:107–112, 1969.
16. Hayes D, Cvitkovic E, Golbey R, et al. Amelioration of renal toxicity of high dose *cis*-platinum diamminedichloride (CPDD) by mannitol-induced diuresis. Proc Am Assoc Cancer Res and ASCO 17:169, 1976.
17. Hrushesky WJ, Levi FA, Halberg F, el al. Cisplatin lethal toxicity is circadian stage-dependent. Cancer Res 42:945–949, 1982.
18. Kociba RJ, and Sleight SD. Acute toxicologic and pathologic effects of *cis*-diamminedichloroplatinum (NSC-119875) in the male rat. Cancer Chemother Rep 55:1–8, 1971.
19. Levi F, Cornelissen G, Nelson W, et al. Cisplatin murine chrononephrotoxicity gauged by two marker rhythms—urine volume and telemetered intraperitoneal temperature. *In* Chronopharmacology (Walker CA, Soliman FKA, and Winget CM, eds). Tallahassee, University Presses of Florida. In press.
20. Levi FA, Hrushesky WJ, Blomquist CH, et al. Reduction of cisplatin nephrotoxicity by optimal circadian drug timing. Cancer Res 42:950–955, 1982.
21. Levi FA, Hrushesky WJ, Halberg F, et al. Lethal nephrotoxicity and hematologic toxicity of *cis*-diamminedichloroplatinum ameliorated by optimal circadian timing. Eur J Cancer. In press.
22. Madias NE, and Harrington JT. Platinum nephrotoxicity. Am J Med 65:307–314, 1978.
23. Nelson W, Tong YL, Lee JK, et al. Methods for cosinor-rhythmometry. Chronobiologia 6:305–323, 1979.
24. Pera MF, Zook BC, and Harder HC. Effects of mannitol or furosemide diuresis on the nephrotoxicity and physiologic disposition of *cis*-dichlorodiammineplatinum (II) in rats. Cancer Res 39:1269–1278, 1979.
25. Rosencweig W, Von Hoff DD, Slavik M, et al. CisDDP (II)—a new anticancer drug. Ann Intern Med 86:803–812, 1977.
26. Shiotsuka RM. Circadian chlorothiazide chronotherapy. PhD thesis. Minneapolis, University of Minnesota, 1976.
27. Simpson HW, Lobban MC, and Halberg F. Urinary near 24-hour rhythms in subjects living on a 21-hour routine in the arctic. Arctic Anthropol 7:144–164, 1970.
28. Sirota J, Baldwin DS, and Villareal H. Diurnal variations of renal function in man. J Clin Invest 29:187–192, 1950.

Toxicology of AMSA in Beagle Dogs and CDF_1 Mice[1,2]

Kenneth L. Pavkov[3,*] and Daryl C. Thake[4,5]

The toxic effects of AMSA in single-dose (×1) or five-daily-dose (×5) regimens were studied in male and female beagle dogs and CDF_1 mice. AMSA was administered orally via gelatin capsules to dogs and as a Klucel suspension to mice. Suitable placebo controls were evaluated. Doses in dogs ranged from 62.5 to 1000 mg/m² (lethal dose [LD]) for the ×1 study and from 31.25 to 500 mg/m² (LD) for the ×5 study. Dogs given the LD (×1 and ×5) had degenerative lesions in the gastrointestinal mucosa, depletion of bone marrow hematopoietic tissue, and lymphoid depletion. These lesions were not seen at doses lower than the LD in the ×1 study but were present in the ×5 study, with severity related to dose. Toxicity appeared to be reversible at the lower doses since animals killed after a 45-day observation period had none of the above lesions. Clinical signs of emesis, diarrhea, and weight loss correlated with the above lesions as did the depressed wbc counts. The toxicity in dogs was dose- and schedule-dependent. The 14-day LD10, LD50, and LD90 values (mg/m²) for mice in the ×1 study were as follows: LD10, 440 in males and 475 in females; LD50, 810 in males and 728 in females; and LD90, 1489 in males and 1117 in females. Mortality was observed initially on Day 4. Single-dose toxicity studies were conducted in mice by oral administration of doses equal to one-half of the LD10, the LD10, and the LD50. The major drug-related lesions in mice included thymic degeneration and atrophy and bone marrow depletion at the higher dose levels, while the major drug-related lesions in dogs included enteric mucosal degeneration and generalized lymphoid and bone marrow depletion. The toxicity of AMSA was reversible in mice and dogs. [Cancer Treat Rep 66:1939–1943, 1982]

The Investigational Drug Branch of the National Cancer Institute (NCI) is presently sponsoring both phase I and II clinical evaluations of AMSA, an acridine derivative (1,2). The promising efficacy of AMSA in the murine tumor models, reported by Cain and Atwell (1) and reviewed by Von Hoff et al (3), has promoted keen interest in this new class of antineoplastic drugs. In preclinical studies of AMSA using an iv formulation, it was found that a solution containing 5 mg/ml of AMSA, 2.8 mg/ml of L-lactic acid, and 100 mg/ml of anhydrous *N*,*N*-dimethylacetamide (DMA) (pH 3.5) or the vehicle alone (equivalent amounts of DMA and L-lactic acid) (pH 3.2) caused overt soreness and local tissue inflammation in guinea pigs and rabbits. The investigators thought the low pH of the formulation was responsible for the irritation (4). Iv infusion of AMSA, formulated as above, in the phase I (3,5,6) and II (7) clinical evaluations has resulted in phlebitis, a major discomforting adverse effect.

Cysyk et al (8) showed that route of administration of AMSA affects the plasma clearance and tissue distribution and that an oral formulation might be desirable. Further, Cysyk suggested that rapid hepatic clearance and biliary excretion of orally administered AMSA might reduce distribution to sensitive tissues and protect against the dose-limiting cytotoxicity associated with iv dosing. Further, orally absorbed AMSA might have target effectiveness against primary or metastasized tumors in the liver.

This preclinical investigation was undertaken to evaluate the toxicity of AMSA administered orally to beagle dogs and CDF_1 mice.

[1]Received Dec 9, 1981; revised June 8, 1982; accepted July 13, 1982.

[2]Supported by contract N01-CM-43746 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The full report is available through the National Technical Information Service, NTIS-No. BCL-KPDT-249992-79-2.

[3]Battelle, Columbus Laboratories, OH.

[4]Monsanto, St. Louis, MO.

[5]We thank Mr. Chris Rahrig and Ms. Barbara Wood for their skillful technical assistance and Dr. Steven Weisbrode, The Ohio State University, for his assistance in pathology.

*_Reprint requests to:_ Dr. Kenneth L. Pavkov, Toxicology and Health Sciences Section, Battelle, Columbus Laboratories, 505 King Ave, Columbus, OH 43201.

MATERIALS AND METHODS

AMSA

AMSA, supplied as bulk powder (lot No. PH41-75-1, sample NA) was obtained from Starks Associates, Inc, Buffalo, NY, through the NCI. The chemical identity of AMSA was verified by infrared spectroscopy, nuclear magnetic resonance, and melting point (228°C–231°C) determination. The purity was estimated to be > 95% since no unidentifiable signals were observed in the nuclear magnetic resonance spectra. The oral dose was prepared fresh daily in gelatin capsules for dogs or suspended (10 mg/ml) in Klucel,[6] with continuous stirring, for oral gavage administration to mice.

Animal Studies

The protocols were from the procedures outlined by Prieur et al (9) and included a toxicity study in mice as described later.

Purebred beagle dogs, 6–7 months of age with mean body weights of 11.7 kg (range, 9.4–13.4) for males and 9.0 kg (range, 7.8–10.0) for females, were supplied by Marshall Research Animals, Inc, North Rose, NY, through the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment (DCT), NCI. The dogs, certified by the breeder to be free of heartworms and immunized against distemper, hepatitis, and leptospirosis, were given a complete physical examination upon receipt and quarantined for a minimum of 2 weeks. These animals were housed individually in stainless-steel cages, fed 400 g of Purina High Protein Dog Chow once each day, and provided water ad libitum. All dogs were identified by a unique permanent numerical tattoo, and the cages were appropriately marked with this number, a study identification, the date of initial treatment, and dose regimen. Fecal examinations for parasites, a minimum of two complete hematologic and serum chemistry profiles, one urinalysis examination, and an ophthalmoscopic examination were performed for each dog prior to initiation of the study. Body weight and rectal temperature were determined a minimum of once per week. After treatment with AMSA, the parameters that were analyzed included hematologic and serum chemistry determinations and urinalyses on Days 2, 4, and 8, and weekly thereafter. Examinations for clinical signs of toxicity were performed daily and final ophthalmoscopic and complete physical examinations were followed by necropsies and histopathologic evaluations. The dogs were treated in pairs, one male and one female at each dose level, according to the treatment schedule shown in table 1.

TABLE 1.—Treatment schedules and dose ranges used for toxicologic evaluation of AMSA administered orally in beagle dogs and CDF_1 mice

Species	Treatment schedule	Dose range (mg/m^2)*
Dogs†	×1	62.5–1000.0
	×5	31.25–500.0
Mice‡	×1	220.5–809.7 (males)
		237.0–728.4 (females)

*Doses in mg/m^2 were estimated by multiplying the mg/kg doses by a factor of 20 for dogs and 3 for mice (ref 10).

†Dose levels for dogs were either doubled or reduced by $^1/_2$ until a lethal dose and a nontoxic dose were established. Toxicity levels (ref 9) for dogs in this study were defined as follows. *Highest nontoxic dose (HNTD)*—the highest dose at which no hematologic, chemical, clinical, or morphologic drug-induced alterations occur; doubling this dose should produce these alterations. *Toxic dose low (TDL)*—the lowest dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose should not produce lethality. *Toxic dose high (TDH)*—the dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces lethality. *Lethal dose (LD)*—the lowest dose which produces drug-induced death during the treatment or observation period.

‡Dose levels for mice were $^1/_2$ the defined LD10, the LD10, and the LD50.

In the single-dose (×1) study in dogs, six pairs were used. Five pairs were treated orally with single doses of 50, 25, 12.5, 6.25, or 3.125 mg/kg (1000, 500, 250, 125, or 62.5 mg/m^2) to obtain the defined toxicity levels (table 2). One pair of dogs was used as a study control and was given a number of empty gelatin capsules equal to the number used to deliver the largest dose. In the repeated-dose (×5) study in dogs, six pairs were used. Five pairs were treated orally with five consecutive daily doses of 25, 12.5, 6.25, 3.125, or 1.562 mg/kg (500, 250, 125, 62.5, or 31.25 mg/m^2) and one pair was given empty gelatin capsules and served as a study control. The most severely affected animal of each dose pair was killed by overdosing with sodium pentobarbital on Day 8 for the ×1 studies and on Day 6 for the ×5 studies. The remaining partner was kept for long-term observation and was killed on Day 46 or Day 52 for the single- or multiple-treatment studies, respectively. All tissues were fixed in 10% neutral buffered Formalin and sections were stained with hematoxylin and eosin for later evaluation. The pituitary, adrenal glands, thyroid, brain, testes or ovaries, liver, kidneys, and heart (left and right ventricles, interventricular septum and atria) of each dog were weighed and ratios of organ to final body weight were computed.

Male and female CDF_1 mice were supplied by Harlan Industries, Inc, and Laboratory Supply Co, Inc, Indianapolis, through the Mammalian Genetics and Animal

[6]0.3% Hydroxypropyl cellulose. Screening Program, Drug Evaluation Branch, NCI.

TABLE 2.—Defined toxicity levels of AMSA administered orally in beagle dogs and CDF_1 mice

Species (sex)	Study	Toxicity*	Dose	
			mg/kg	mg/m^2
Dog (M,F)	×1	LD	50.0	1000.0
		TDH	25.0	500.0
		TDL	6.25	125.0
		HNTD	3.125	62.5
Dog (M,F)	×5	LD	25.0	500.0
		TDH	12.5	250.0
		TDL	3.125	62.5
		HNTD	1.562	31.25
Mouse (M) 14-day lethality		LD90	496.2	1488.6
		LD50	269.9	809.7
		LD10	146.8	440.4
Mouse (F) 14-day lethality		LD90	372.4	1117.2
		LD50	242.8	728.4
		LD10	158.3	474.9

*See table 1 for definitions.

Production Section, DCT, NCI. Mice were quarantined for a minimum of 1 week prior to test. Mice were housed in polycarbonate cages with chipped hardwood bedding. Purina Rodent Chow 5001 and water were available ad libitum except during the 6-hour predosing fast when only food was withheld. In the lethality study, single oral gavage doses were administered to ten animals of each sex (21–24 g of body weight) and the mice were observed for 14 days. One-half of the determined LD10, the LD10, and the LD50 doses and Klucel (vehicle) and untreated control groups were used to determine drug toxicity in six mice per sex per dose. All animals treated with the LD50 dose were killed on Day 3 (Day 1 was the day of treatment) along with one-half of the mice in each of the other study groups. The remaining animals were killed on Day 15 (14th observation day). The toxicity end points included clinical signs and body weights during the observation period and hematologic and selected serum chemistry analyses (BUN, SGOT, and SGPT) on the termination day. Necropsies and histopathologic evaluations were performed on each animal.

RESULTS

Single-Dose Study in Dogs

Clinical response.—The male treated with 50 mg/kg died on Day 5, whereas the female, after showing the toxic signs described below, returned to a clinically normal condition and survived until the scheduled termination on the 46th test day. Within 3 hours after treatment, both animals at the highest dose had a 3°F drop in rectal temperature, and the female had mucoid (orange-colored) stool. In the male, emesis, depression, anorexia, diarrhea, and a 19% loss of initial weight were observed during the first 5 days. The female had mucoid diarrhea on Days 3, 30, and 31 but did not show other signs of toxicity. Mucoid diarrhea, soft stool, emesis, and slight weight loss were observed in dogs from nonlethal dose groups, but these effects did not appear to be dose-related. Leukopenia was dose-related at all the toxic dose levels with nadirs during the first week after treatment. Dogs kept for extended observation quickly returned to normal. Reticulocytopenia was observed during the first week after treatment in dogs treated at the 50-, 25-, and 12.5-mg/kg dose levels and returned to normal in the second week. Although this response was extensive at the two highest doses, the number of circulating erythrocytes was not dramatically affected, and nucleated rbcs were never observed in the peripheral blood samples.

Pathology.—Tissue changes that were attributed to a toxic drug effect occurred mainly in the one dog that died on Day 5 following treatment with 50 mg/kg. In the small intestine the lesions were characterized by mucosal degeneration with crypt cell changes indicative of cell cycle alterations. Congestion in the mucosa was present in the ileum and colon, and hemorrhage occurred in the jejunal mucous membrane. Degenerative changes were also apparent in the glandular epithelium of the esophagus in this animal. A generalized lymphoid depletion of moderate to severe intensity involved all lymphoid organs examined (spleen, lymph nodes, thymus, and Peyer's patches). The hematopoietic elements of the bone marrow were also severely depleted. Centrilobular regions of the liver were markedly congested with mild necrosis. Other lesions included moderate hemorrhage in the mesenteric lymph node and moderate vacuolation of the cells of the zona glomerulosa

and zona fasciculata of the adrenal cortex. The only drug-related lesions in the high-dose animal held for extended observation occurred in the liver and included diffuse vacuolation of centrilobular hepatocytes and dilation of the portal lymphatic vessels. Other treatment-related lesions were observed in dogs from the intermediate dose level: 12.5 mg/kg. The female killed on Day 8 had mild but generalized erythrophagocytosis and hemorrhage in the lymph nodes and the male killed on Day 46 had mild centrilobular hepatic necrosis. There were no drug-related lesions observed in dogs treated with 25, 6.25, or 3.125 mg/kg of AMSA. Organ weights (absolute and relative to final body weight) were within normal limits.

Five Daily Doses in Dogs

Clinical response.—The highest dose level, 25 mg/kg, was lethal to the female on Day 6 and to the male on Day 7. Toxic effects observed in this treatment regimen were very similar to those seen in the single-dose treatment. Depression and lethargy were observed toward the end of the treatment period in both dogs given the 25-mg/kg dose. Diarrhea and emesis were seen on the third through fifth treatment days (6–24 hours after treatment) at the two highest dose levels. Body weights decreased by 12%–13% on Day 6 in the lethal-dose dogs and by 7%–9% in dogs at the 12.5-mg/kg dose. The body weight of the male held for extended observation (47-day recovery period) returned to near normal by Day 52. The manifestations of clinical signs at the lower doses were not consistently dose-related. The female treated with 6.25 mg/kg daily for 5 days had mucoid diarrhea on treatment Days 3, 4, and 5 and diarrhea on Day 6. Although there were no clinical signs of drug toxicity such as emesis or diarrhea at the 3.125-dose level, the female treated with 1.562 mg/kg/day for 5 days had emesis on Days 1 and 3 and diarrhea on Days 3 and 5. Decreases in reticulocyte and leukocyte populations were observed at all dose levels. Severity, time of onset, and duration of response for these parameters were directly related to dose level. Rapid reversibility of these effects was seen in all animals held for the extended observation period. At the 25-mg/kg dose level there was evidence of hemoconcentration (hematocrit of 54%) in the male prior to death. There were slightly elevated BUN values (increases to 25 and 23 mg/100 ml in the male and female, respectively) just prior to death, and the SGOT was increased to 33 IU in the female on the second day of treatment. At the lower dose levels, neutropenia and lymphopenia were observed. Decreases in circulating myeloid and erythroid elements occurred in conjunction with depleted bone marrow in the female at the 12.5-mg/kg dose level. The male from this dose group had decreased hematocrit and hemoglobin values and decreased reticulocyte, erythrocyte, and leukocyte counts during and immediately following the 5-day treatment period. In a dose-related fashion, these parameters were depressed and returned to normal during the 47-day observation period, indicating the reversibility of the bone marrow suppression. Serum chemistry values fluctuated mildly at the lower dose levels but did not indicate toxic effects.

Pathology.—The dogs that received 25 mg/kg/day for 5 days died on Days 6 and 7 and had lesions similar to those described for the male dog treated with a single 50-mg/kg dose of AMSA. Mucosal degeneration of the small intestine and colon, moderate to severe lymphoid depletion or necrosis in most lymphoid tissues, and severe bone marrow depletion were present. At the lower dose levels, the severity of these lesions was related to dose level and occurred in the animals killed on the day following the fifth and last treatment. No drug-related pathologic lesions were observed in animals killed following the 47-day observation period. Organ weights were within normal limits.

Median Lethal Dose in Mice

For CDF_1 mice, the 14-day, oral LD50 was found to be 269.9 mg/kg (809.7 mg/m^2) for males and 242.8 mg/kg (728.4 mg/m^2) for females. The 95% confidence intervals were 237.4–306.8 mg/kg and 213.2–276.4 mg/kg, respectively. The LD10 was 146.8 mg/kg (440.4 mg/m^2) for males and 158.3 mg/kg (474.9 mg/m^2) for females, with 95% confidence intervals of 100.8–213.7 mg/kg and 118.0–212.2 mg/kg, respectively. The median lethal doses were calculated for combined sexes for comparison with previously published data and are shown in table 3. Mortality occurred from Day 4 through Day 8 in males and from Day 4 through Day 9 in females. For both sexes the majority of deaths at each dose level occurred from Day 4 through Day 6. The modal-day of death for each sex was Day 5. Piloerection and lethargy were the dose-related clinical signs of toxicity through Day 10.

Single-Dose Acute Toxicity in Mice

All animals treated at the defined male and female LD50 dose levels were killed on the third day of the study with no apparent clinical abnormalities. Treatments at the LD50, the LD10, and one-half the LD10 suppressed the hematopoietic tissues of mice as shown in the abnormal hematologic parameters in animals killed on test Days 3 and 15. Toxic signs at the lower doses included weight loss, piloerection, and lethargy from Days 4 through 10. The male mice given the LD10 dose and not killed on Day 3 all died on Day 8. One female at the LD10 dose level died on Day 6 and had severe degeneration and necrosis of the thymus and severe atrophy of the bone marrow. The histopathologic eval-

TABLE 3.—Comparative lethal single-dose levels of oral and iv AMSA in CDF_1 mice

Route	Defined toxicity levels (mg/m^2)*		
	LD90	LD50	LD10
Oral	1270.2 (1105.8–1458.9)	761.4 (704.7–822.9)	456.6 (375.3–555.3)
Iv†	111.9 (107.7–116.4)	101.1 (98.7–103.5)	91.2 (87.3–95.7)

*Values in parentheses = 95% confidence intervals.
†Ref 4 and Henry et al (Oct 20, 1976) Report No. IITRI-Tox-249992-76-2 to DHEW-PHS, NIH-NCI, Laboratory of Toxicology, Bethesda, MD 20205.

TABLE 4.—Comparative toxic dose levels of oral and iv AMSA in beagle dogs

Study	Route	Defined toxicity levels (mg/m^2)			
		LD	TDH	TDL	HNTD
×1	Oral	1000.0	500.0	125.0	62.5
	Iv*	125.00	62.60	31.24	15.62
×5	Oral	500.0	250.0	62.5	31.25
	Iv*	15.60	7.80	3.90	1.96

*Ref 4.

uations of mice killed on Day 3 showed mild to marked lymphoid depletion of the cortical thymus and mild to severe atrophy of the bone marrow. These lesions were not seen in mice given the LD10 (female) or one-half of the LD10 (male or female) and killed on Day 15.

DISCUSSION

Our studies indicated that the oral administration of AMSA induced toxic changes to bone marrow, lymphoid tissue, intestinal mucosa, and liver. These toxic effects were similar to those reported by Henry et al (4) for iv administered AMSA. However, we did not observe CNS or renal toxic effects either by clinical or pathologic evaluations. This might be expected since earlier studies by Cysyk et al (8) showed that the oral route of administration resulted in considerably lower levels of unchanged AMSA in kidney and brain tissues compared to levels found following iv administration. As shown in tables 3 and 4, using the same definition of toxicity, the tolerable dose levels in our study are higher than those reported earlier for iv administration. It may be possible to direct higher doses of AMSA to specific tumor sites in clinical studies using the oral route of administration. This approach would avoid iv infusion and the associated phlebitis.

REFERENCES

1. CAIN BF, and ATWELL GJ. The experimental antitumor properties of three congeners of the acridylmethanesulphonanilide (AMSA) series. Eur J Cancer 10:539–549, 1974.
2. CAIN BF, WILSON WR, and BAGULEY BC. Structural-activity relationships for thiolytic cleavage rates of antitumor drugs in the 4′-(9-acridinylamino)methanesulfonanilide series. Mol Pharmacol 12:1027–1035, 1976.
3. VON HOFF DD, HOWSER D, GORMLEY P, ET AL. Phase I study of methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl] -(*m*-AMSA), using a single-dose schedule. Cancer Treat Rep 62:1421–1426, 1978.
4. HENRY MC, PORT CD, and LEVINE BS. Preclinical toxicologic evaluation of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) in mice, dogs, and monkeys. Cancer Treat Rep 64:855–860, 1980.
5. LEGHA SS, GUTTERMAN JU, HALL SW, ET AL. Phase I clinical investigation of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (NSC 249992), a new acridine derivative. Cancer Res 38:3712–3716, 1978.
6. RIVERA G, EVANS WE, DAHL GV, ET AL. Phase I clinical and pharmacokinetic study of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide in children with cancer. Cancer Res 40:4250–4253, 1980.
7. LEGHA SS, LATREILLE J, MCCREDIE KB, ET AL. Neurologic and cardiac rhythm abnormalities associated with 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) therapy. Cancer Treat Rep 63:2001–2003, 1979.
8. CYSYK RL, SHOEMAKER DD, AYERS OC, ET AL. Oral absorption and selective tissue localization of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide. Pharmacology 16:206–213, 1978.
9. PRIEUR DJ, YOUNG DM, DAVIS RD, ET AL. Procedures for preclinical toxicologic evaluation of cancer chemotherapeutic agents: protocols of the laboratory of toxicology. Cancer Chemother Rep Part 3, vol 4(1):1–30, 1973.
10. FREIREICH EJ, GEHAN EA, RALL DP, ET AL. Quantitative comparison of toxicity of anticancer agents in mouse, rat, dog, monkey, and man. Cancer Chemother Rep 50:219–244, 1966.

Activity of 1-*p*-(3,3-Dimethyl-1-triazeno) Benzoic Acid Potassium Salt in M 5076/73A Ovarian Reticular Cell Sarcoma of the Mouse[1,2]

T. Colombo,[3] S. Garattini,[3] L. Lassiani,[4] and M. D'Incalci[3,*]

1-*p*-(3,3-dimethyl-1-triazeno) benzoic acid potassium salt (DM-COOK) was tested on M 5076/73A (M5) mouse sarcoma at a dose of 40 or 50 mg/kg/day (Days 6–14) after im transplant of 7×10^5 cells, with or without surgical removal of the primary tumor on Day 14. Treatment at either dose level resulted in reduction of the primary tumor weight to around 50% of that in the controls, and striking antimetastatic effects were observed. When a dose of 40 or 50 mg/kg of DM-COOK was followed by surgery, there were 14% and 40% long-term survivors, respectively, but the higher dose caused about 30% toxic deaths. After iv injection of 10^3 or 10^5 M5 tumor cells, no artificial metastases appeared in DM-COOK-treated mice, whereas all control animals had metastatic involvement in the liver, spleen, ovaries, and kidneys. [Cancer Treat Rep 66:1945–1948, 1982].

Aryl-dimethyltriazenes (DMTs) are antitumor agents that have shown activity in a number of experimental tumors in the mouse (1,2). They were initially developed with the idea that dacarbazine, which is clinically used for treatment of melanoma and soft tissue sarcoma (3–5), could be replaced by other DMTs with greater stability and forming fewer diazonium ions, which are considered to be responsible for toxicity but not for antitumor activity (6).

The mechanism of action of DMTs is still unknown, but one possibility is that it is mediated by the monomethyltriazenes, potent alkylating agents formed by oxidative *N*-demethylation of the parent compound (7). Some other bioproducts of DMTs, however, may have more selective action against tumors sensitive to these compounds (8). In addition it has been suggested that some DMTs exert an antimetastatic effect independently of their cytotoxic effect. Sava et al[4](9) reported that 1-*p*-carboxamidophenyl-3,3-dimethyltriazene and 1-*p*-carboxyphenyl-3,3-dimethyltriazene and its potassium salt DM-COOK displayed a selective antimetastatic effect on Lewis lung carcinoma; no lung metastases were found after DM-COOK treatment. Conversely, when these tumor cells were injected iv, DM-COOK had no effect on the artificial metastases growing in the lung, suggesting that the antimetastatic effect was not directly related to its cytotoxic properties (9,10). The authors therefore speculated that the compound might act by preventing metastatic dissemination.

In this study in the mouse we investigated the antitumor and antimetastatic effects of DM-COOK, with and without surgery of the primary tumor, on the ovarian reticular cell sarcoma M 5076/73A (M5), which metastasizes preferentially to peritoneal organs (11,12).

MATERIALS AND METHODS

Animals and Tumor

Female C57BL/6J mice (20 ± 2 g) obtained from Charles River Laboratories, Italy, received an im transplant of 7×10^5 M5 cells. The M5 ovarian sarcoma was supplied by Mason Research Institute, Division of Cancer Treatment, Animals and Human Tumor Bank, Worcester, MA. Artificial metastases were obtained by iv injection of 10^3 or 10^5 tumor cells.

Treatment

The animals were injected ip with 0.1 ml/10 g of the drug freshly prepared in the dark as a solution in 0.1 M $NaHCO_3$. Treatment was given daily for 9 consecu-

[1]Received Dec 29, 1981; revised July 8, 1982; accepted Aug 5, 1982.
[2]Supported in part by Public Health Service grant CA-12764 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and CNR contract No. 80.01539.96. The generous contribution of the Italian Association for Cancer Research is gratefully acknowledged.
[3]Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.
[4]Istituto di Chimica Farmaceutica, Università di Trieste, Italy.
Reprint requests to: M. D'Incalci, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.

TABLE 1.—Antitumor and antimetastatic activity of DM-COOK (ip), with or without surgery, in mice bearing M5 ovarian sarcoma*

Dose (mg/kg)	Surgery	Primary tumor weight at surgery in g (mean ± SE)	Toxic deaths (%)	Animals with metastases at death (%)			T/C (%)†	Long-term survivors (%)‡
				Liver	Ovaries	Spleen		
—	No	—	—	100	18	0	—	0
40	No	—	0	18	0	0	148	0
—	Yes	1.75 ± 0.10	—	100	40	7	—	0
40	Yes	0.70 ± 0.03§	0	7	0	0	250	14
—	No	—	—	100	7	0	—	0
50	No	—	33	20	7	0	100	0
—	Yes	1.44 ± 0.05	—	100	27	7	—	0
50	Yes	0.68 ± 0.02§	27	0	0	0	132	40

*Each group consisted of 15 mice. Treatment was given daily for 9 consecutive days, starting on Day 6 after tumor transplantation. Surgery was carried out on Day 14 after tumor transplantation.

†T/C (%) = median survival time of treated mice/median survival time of untreated controls, × 100.

‡% treated animals surviving > 150 days after tumor implantation.

§$P < 0.01$ vs respective controls (Student's t test).

tive days, starting on Day 6 after tumor implantation. Control animals received the vehicle only.

Surgical Tumor Removal

The tumor was removed surgically 14 days after transplantation. Animals were anesthetized with ether, an incision was made above the tumor, the femoral artery and adjacent blood vessels were tied with sterile surgical silk thread, and the tumor-bearing leg was removed. The wound was washed with sterile normal saline and closed with Michell 7-mm wound clips. No mortality, no primary tumor recurrence, and no bacterial infections were observed after surgery.

Evaluation of Effects

In one experiment, autopsy was performed on Day 37 after transplant; in the others, autopsy was performed within 12 hours of death. The tumor weight was assessed by weighing the entire leg with the tumor. The weight of the tumor was not considered in experiments without surgical treatment, because cannibalism made it impossible to assess it with sufficient accuracy. Metastases were assessed by weighing the organs with metastases and by gross inspection. The median survival time was calculated as described by Geran et al (13). Death was classified as toxic death when it occurred before the first death among untreated control animals.

RESULTS

Table 1 reports the effect in mice bearing M5 ovarian reticular cell sarcoma of DM-COOK at daily doses of 40 or 50 mg/kg for 9 days starting on Day 6 after tumor transplantation, with or without surgery. At both dose levels DM-COOK showed a striking antimetastatic effect, causing reduction of the primary tumor and prolonging survival time, particularly when the treatment was followed by surgical eradication of the primary tumor. The 50-mg/kg dose appeared to be very active, inducing 40% cures in the mice from which the tumor was removed, but approximately 30% toxic deaths were observed. The 40-mg/kg dose was much better tolerated and showed similar activity on primary and secondary

TABLE 2.—Antitumor and antimetastatic activity of DM-COOK (ip), with surgery, in mice bearing M5 ovarian sarcoma*

Dose (mg/kg)	Weight (mean ± SE)			Animals with metastases (%)		
	Liver (g)	Ovaries (mg)	Spleen (mg)	Liver	Ovaries	Spleen
—	1.37 ± 0.297	67.9 ± 21.6	154 ± 13	54	100	0
40	0.646 ± 0.006	9.2 ± 1.3†	94 ± 12‡	0	0	0

*Each group consisted of 10 mice. Treatment was given daily for 9 consecutive days, starting on Day 6 after tumor transplantation. Surgery was carried out on Day 14 after tumor implantation (tumor weight at this time was 1.10 ± 0.05 g for controls and 0.61 ± 0.02‡ g for treated animals). Antimetastatic activity was evaluated 37 days after tumor transplantation.

†$P < 0.05$ vs controls.

‡$P < 0.01$ vs controls.

TABLE 3.—Effect of DM-COOK on artificial metastases*

No. of tumor cells injected iv/mouse	Dose (mg/kg, ip)	Weight (mean ± SE)				Animals with metastases (%)			
		Liver (g)	Ovaries (mg)	Spleen (mg)	Kidneys (mg)	Liver	Ovaries	Spleen	Kidneys
10^3	—	1.708 ± 0.145	17 ± 3	259 ± 22	206 ± 9	100	7	93	14
10^3	40	0.966 ± 0.044†	14 ± 1	184 ± 13†	203 ± 7	0	0	0	0
10^5	—	1.886 ± 0.147	27 ± 3	265 ± 21	202 ± 7	100	71	100	93
10^5	40	1.078 ± 0.062†	12 ± 1†	162 ± 12†	210 ± 5	0	0	0	0

*Each group consisted of 14 mice. Animals were killed and examined for metastases on Day 18 from tumor cell injection.
†$P < 0.01$ vs respective controls (Student's t test).

tumors. A considerable number of late deaths from undetermined cause (81–118 days after tumor implantation) were observed among animals treated with DM-COOK and surgery. We noted only a weight loss (6–8 g) in the animals before death and found no macroscopic neoplastic lesions at autopsy.

Table 2 shows the results of DM-COOK treatment with doses of 40/mg/kg for 9 days before surgery, assessed by autopsy on Day 37 after tumor transplantation. This experiment confirms the clear-cut antimetastatic effect of DM-COOK in M5-bearing mice, as all the animals were apparently free of metastatic involvement in liver and ovaries, whereas these organs had metastases in most control animals. The drug also induced regression of the primary malignancy, as assessed by weighing the tumor removed at surgery. To shed some light on the antimetastatic mechanism of DM-COOK we evaluated the drug's effect on artificial metastases obtained by iv injection of 10^3 or 10^5 cells. As shown in table 3, no metastases appeared in DM-COOK-treated animals, whereas all animals of both control groups had metastatic involvement of the liver, spleen, ovaries, and the kidneys.

The unexpectedly large weight of the spleen in the experiments of treated animals without macroscopic metastases, contrasting with the finding of the previous experiment (table 2), prompted us to investigate whether DM-COOK treatment influenced the size of the organs evaluated in this study. We found that in non-tumor-bearing mice, spleen weight was significantly increased 18 days after the beginning of DM-COOK treatment with 9 daily doses of 40 mg/kg (60 ± 2 mg for controls vs 92 ± 5 mg for treated animals; $P < 0.01$). However, 37 days after the start of treatment, spleen weights were normal again (71 ± 2 mg for controls vs 72 ± 2 mg for treated animals).

The weights of liver, kidney, intestine, and ovary were unchanged 18 and 37 days after the start of treatment.

DISCUSSION

DM-COOK appears to be an active antitumor agent against mouse M5 reticular cell sarcoma of the ovary, showing a more marked effect on metastatic lesions than on the primary tumor. This was clear in all of the studies described, but when treatment was followed by surgery, a conspicuous increase in survival time was observed. These data are consistent with previous reports of the antimetastatic properties of this compound in another murine transplantable tumor, Lewis lung carcinoma (3LL), which results in metastatic deposits in the lung (9,10).

Metastases have been observed to have greater sensitivity than primary tumor in studies of several other drugs; this may be related to the faster kinetic rate of metastatic cells (14–15) and to the greater availability of the drug or its active metabolites (16).

Giraldi et al (10) claim that, in 3LL, DM-COOK has selective antimetastatic properties likely to be related to the inhibition of dissemination mechanisms. In our experimental conditions, however, DM-COOK showed a definite cytotoxic activity against M5 tumor cells. This is consistent with the reported cytotoxic activity of other phenyl-dimethyltriazenes (6) and is likely to be related to the formation of reactive metabolites; monomethyl derivatives, for example, are potent methylating agents (6,7).

The difference between our findings and Giraldi's may be due to the lower sensitivity of 3LL cells to the cytotoxic action of DM-COOK; they may become susceptible only when a relatively low number of cells disseminate into the bloodstream, becoming directly exposed to the drug or its active metabolites. This explanation is also suggested by previous experiments in this laboratory in which iv injection of 2×10^5 3LL cells was followed by a scant inhibitory effect on the formation of lung metastases, but a 50% reduction was evident after injection of 5×10^4 cells.

DM-COOK as a cytotoxic compound appears to be worthy of further investigation in other experimental tumors because it has striking antitumor activity, at least in the M5 tumor. The observed spleen enlargement caused by DM-COOK and the late deaths without evidence of disease are two points that also require further study.

REFERENCES

1. Audette RCS, Connors TA, Mandel HG, et al. Studies on the mechanism of action of the tumour inhibitory triazenes. Biochem Pharmacol 22:1855–1864, 1973.
2. Giraldi T, Nisi C, Connors TA, et al. Preparation and antitumor activity of 1-aryl-3,3-dimethyltriazene derivatives. J Med Chem 20:850–853, 1977.
3. Carter SK, and Friedman MA. 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC, DIC, NSC-45388)—a new antitumor agent with activity against malignant melanoma. Eur J Cancer 8:85–92, 1972.
4. Comis RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165–176, 1976.
5. Beretta G, Bonadonna G, Bajetta E, et al. Combination chemotherapy with DTIC (NSC-45388) in advanced malignant melanoma, soft tissue sarcomas, and Hodgkin's disease. Cancer Treat Rep 60:205–211, 1976.
6. Connors TA, Goddard PM, Merai K, et al. Tumour inhibitory triazenes: structural requirements for an active metabolite. Biochem Pharmacol 25:241–246, 1976.
7. Vaughan K, and Stevens MFG. Monoalkyltriazenes. Chem Soc Rev 7:377–397, 1978.
8. Gescher A, Hickman JA, Simmonds RJ, et al. Studies of the mode of action of antitumour triazenes and triazines II. Investigation of the selective toxicity of 1-aryl-3,3-dimethyltriazenes. Biochem Pharmacol 30:89–93, 1981.
9. Sava G, Giraldi T, Lassiani L, et al. Mechanism of the antimetastatic action of dimethyltriazenes. Cancer Treat Rep 63:93–98, 1979.
10. Giraldi T, Sava G, Cuman R, et al. Selectivity of the antimetastatic and cytotoxic effects of 1-*p*-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt, (±)-1,2-di(3,5-dioxopiperazin-1-yl)propane, and cyclophosphamide in mice bearing Lewis lung carcinoma. Cancer Res 41:2524–2528, 1981.
11. Alessandri G, Giavazzi R, Falautano P, et al. A murine ovarian tumor with unique metastasizing capacity. Eur J Cancer 17:651–658, 1981.
12. Hart IR, Talmadge JE, and Fidler IJ. Metastatic behavior of a murine reticulum cell sarcoma exhibiting organ-specific growth. Cancer Res 41:1281–1287, 1981.
13. Geran RI, Greenberg NH, Macdonald MM, et al. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (3rd edition). Cancer Chemother Rep Part 3, vol 3(2):1–103, 1972.
14. DeWys WD. A quantitative model for the study of the growth and treatment of a tumor and its metastases with correlation between proliferative state and sensitivity to cyclophosphamide. Cancer Res 32:367–373, 1972.
15. Schabel FM, Jr. Concepts for systemic treatment of micrometastases. Cancer 35:15–24, 1975.
16. Donelli MG, Colombo T, Broggini M, et al. Differential distribution of antitumor agents in primary and secondary tumors. Cancer Treat Rep 61:1319–1324, 1977.

Hematopoietic Stem Cells in Friend Murine Leukemia Virus-Infected Mice Undergoing Chemotherapy: Effects of 5-FU[1,2]

H. J. Seidel* and Ludwika Kreja[3,4]

Uninfected DBA/2 mice and those infected 12 days previously with the polycythemia-inducing Friend murine leukemia virus were treated with 5-FU in doses of 18.75–200 mg/kg ip, and the effect on spleen weight, reticulocytosis, and hematopoietic stem cell compartments CFU-S, CFU-C, and CFU-E was studied. The CFU-E population of virus-infected mice, which had been completely independent of erythropoietin (Ep) in vitro before treatment, showed a considerable proportion of normal Ep-requiring CFU-E at Days 2–6 after doses ≤ 50 mg/kg, indicating a chemotherapy-induced remission. Later, Ep-independent growth was seen again, most probably due to a reinfection by the virus at the target cell level. The regeneration of all stem cell types was quicker in uninfected mice than in those infected with virus. These data are discussed in relation to results obtained with hydroxyurea treatment, where Ep-dependent CFU-E were also seen, and with respect to the effects of busulfan, carmustine, and dactinomycin, with their different modes of action. The importance of effective antiviral therapy for the cure of such a virus-induced malignancy is emphasized. [Cancer Treat Rep 66:1949–1956, 1982]

The chemotherapy of leukemia has two goals that are both indispensable for a complete remission and cure from the disease: elimination of the leukemic cell population and re-establishment of a normally functioning hematopoietic cell system.

The study of these parameters is facilitated if the leukemic cells can be quantified, eg, by use of a marker. In the past we have shown in the experimental Friend murine leukemia (polycythemia) model that the apparent erythropoietin (Ep)-independent growth of the Friend virus-transformed erythroid cell system can be used as such a marker; only transformed erythroid-committed precursors (CFU-E) form colonies in vitro without addition of Ep (1–5). In a study using multiple doses of hydroxyurea, we were able to kill all Ep-independent CFU-E; during the regeneration of the hematopoietic cell system, which started at a pluripotent stem cell (CFU-S) level that was 20% of that of controls, a wave of normal Ep-dependent CFU-E was seen, indicating the reinduction of normal hematopoiesis (6).

This finding was interpreted in accordance with the idea of a structuredness of the stem cell pools as proposed by Rosendaal et al (7): that previously resting and non-virus-infected stem cells survived the treatment and gave rise to this normal, Ep-dependent erythropoiesis in which, however, cells were reinfected by the virus after some days. In the studies presented in this paper we used 5-FU. This drug inhibits thymidylate synthetase and thus DNA synthesis, but in contrast to hydroxyurea, its killing effect is not restricted to cells in the S-phase (8). Rapidly proliferating stem cells like CFU-E and colony-forming units in culture (CFU-C) are more sensitive than CFU-S; a review of 5-FU action on hematopoietic stem cells is included in the article by Marsh (9). More recent studies have shown that a high dose (150 mg/kg) eliminates all proliferating committed cells and spares just a noncycling stem cell with a very high repopulating ability (10). If this stem cell is not virus-infected and enaoles a rapid regeneration, the effect on the Friend leukemia and the Ep-independent erythropoiesis should be similar to that described for hydroxyurea treatment.

MATERIALS AND METHODS

Virus

N-tropic polycythemia-inducing Friend murine leu-

[1]Received Dec 30, 1981; revised July 9, 1982; accepted July 26, 1982.
[2]Supported by the Deutsche Forschungsgemeinschaft (SFB 112).
[3]Department of Clinical Physiology, University of Ulm, W Germany.
[4]We thank E. Barthel and K. Steinhoff for their technical assistance.
**Reprint requests to:* Dr. H. J. Seidel, Department of Clinical Physiology, University of Ulm, Oberer Eselsberg, 7900 Ulm, W Germany.

kemia virus (F-MuLV-P) was provided by R. A. Steeves (Albert Einstein Medical College, New York). It was serially passaged ip in DBA/2 mice with 0.25 ml of a 10% cell-free homogenate of leukemic spleens (2–3 g) in saline.

Mice

Female DBA/2 and BDF_1 mice (Gl. Bomholtgard, Ry, Denmark) weighing 19–22 g were used. The mice were kept ten to a cage in artificial light for 12 hrs daily. They were fed commercial pellets and water ad libitum.

Experimental Procedures

For each experiment, four to six mice were used. The mice were killed by cervical dislocation, their spleen weights were determined, and a single-cell suspension of pooled spleen or femur marrow was prepared in α-medium (Flow Laboratories, Inc, Rockville, Md) containing 2% fetal calf serum (Seromed, Munich). Appropriate cell concentrations were obtained by dilution and the cells were counted in a Coulter counter. The total spleen cellularity was calculated according to Wendling et al (11), 100 mg corresponding to 10×10^7 cells.

Blood for hematologic studies was taken from the retro-orbital sinus and studied by standard procedures. Reticulocytes were stained with new methylene blue and counted.

CFU-S Experiments

The procedure described by Till and McCulloch (12) was used to assay multipoint hematopoietic stem cells. The pooled marrow or spleen cells were injected iv ($2–6 \times 10^4$ marrow cells and $2–6 \times 10^5$ spleen cells in 0.25 ml) into irradiated mice (ten animals/group). Irradiation consisted of an 800-rad exposure at 280 kV and 12 mA, with a 1.4-mm Cu and 1-mm Al filter, a focal distance of 50 cm, and a rate of 30 R/min. The recipients were killed 9 days after injection of cells, and their spleens were removed and placed in Bouin's solution in preparation for counting macroscopic colonies after 24 hrs in the fixative. From these data, the mean CFU-S content per femur or spleen was calculated. No endogenously formed colonies were found in mice exposed to radiation but not grafted with cells.

CFU-C Experiments

Cells from bone marrow or spleen were incubated in 3.5-cm plastic dishes containing 1 ml of α-medium with 20% horse serum (Seromed) and 0.3% agar. Colony growth was stimulated by the use of heat-inactivated serum obtained from NMRI mice 3 hrs after they received iv injections of 50 μg of endotoxin (*Salmonella abortus equi;* Difco Laboratories, Detroit). Optimal stimulation was obtained by the addition of 12.5 or 25 μl of endotoxin-activated serum. The cell number per plate was $0.4–2 \times 10^5$ for bone marrow cells and $0.5–2 \times 10^6$ for spleen cells. The cultures were done in triplicate and incubated in 5% CO_2 at 37°C in a humidified atmosphere. After 7 days of incubation, the colonies (> 50 cells being a colony) were counted with the use of 40-fold magnification. In each experiment, a control culture with normal bone marrow and spleen cells was included. The number of colonies per dish varied about 10% of the mean.

CFU-E Experiments

The method described by Iscove and Sieber (13) was used. The following were mixed in a final volume of 2.5 ml: 0.8% methylcellulose; 30% fetal calf serum; Ep step III (Connaught Medical Research Laboratories, Toronto), 0.2 or 0.3 units/ml, depending on the batch; α-thioglycerol at an end concentration of 10^{-4} M; and bone marrow or spleen cells in α-medium. Then 1 ml with 3×10^5 cells was plated into a 3.5-cm plastic petri dish (Greiner & Co, Nürtingen, Federal Republic of Germany). For each experiment, four parallel cultures were set up. Colonies were counted after 48 hours' incubation at 37°C in a humidified atmosphere containing 5% CO_2. Erythroid colonies with more than eight small cells (erythroblasts) were scored without staining at an 80-fold magnification.

Cultures from F-MuLV-P-infected mice were also set up without addition of Ep, in order to see the F-MuLV-P-specific Ep-independent CFU-E growth (1–3). Ep independence of the complete erythroid precursor compartment was identified when the same number of colonies was seen in the plates with and without addition of Ep and filled with the same cell suspension from an F-MuLV-P-infected animal.

The variation in the number of colonies per plate filled with the same cell suspension did not exceed 10%. As in the controls, day-to-day variations in the yield of erythroid colonies were found, generally dependent on the different batches of methylcellulose used and changes in pH during the preparation of the cell suspension and cultivation in the incubator. Therefore, parallel cultures from uninfected controls were plated for each experiment.

5-FU Treatment

5-FU (Hoffmann-La Roche) was dissolved in saline and the doses indicated were injected ip after dilution in 0.2 ml.

RESULTS

Effect of 5-FU Treatment on Spleen Weight and Reticulocytosis

Groups of DBA/2 mice were infected with F-MuLV-P and received a single 37.5–200-mg/kg dose of 5-FU 12 days later. Spleen weight and reticulocytosis were reduced as shown in figures 1 and 2, respectively. There was a clear dose-dependent effect, with a progressive recovery of both parameters from Day 7 on. After a dose of 37.5 mg/kg, the regeneration was almost completed at this time. In addition, bone marrow cellularity and hematocrit were determined. Bone marrow cellularity recovered parallel to the increase of the spleen weight. The hematocrit of untreated F-MuLV-P-infected mice rose from 61% at the beginning of the experiment to 77% at Day 14; after 5-FU treatment it fell in a dose-dependent manner to 44%–57% at Day 7 and 40%–76% at Day 14.

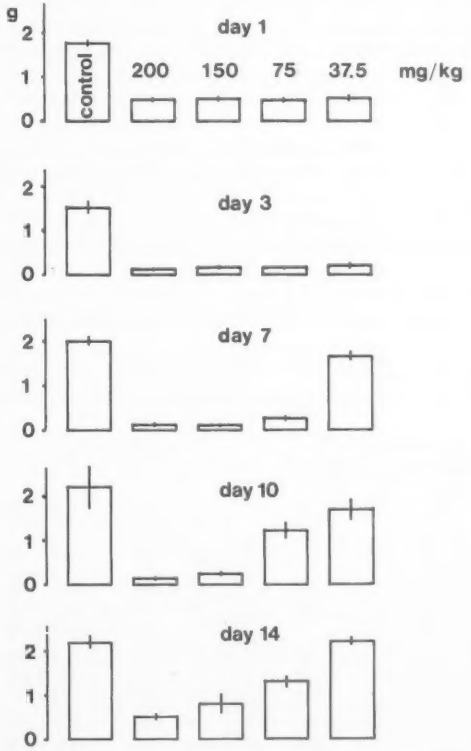


FIGURE 1.—Effect of a single dose of 5-FU ip on spleen weight of F-MuLV-P-infected DBA/2 mice. Treatment was given 12 days after virus infection. Vertical bars indicate SEM.

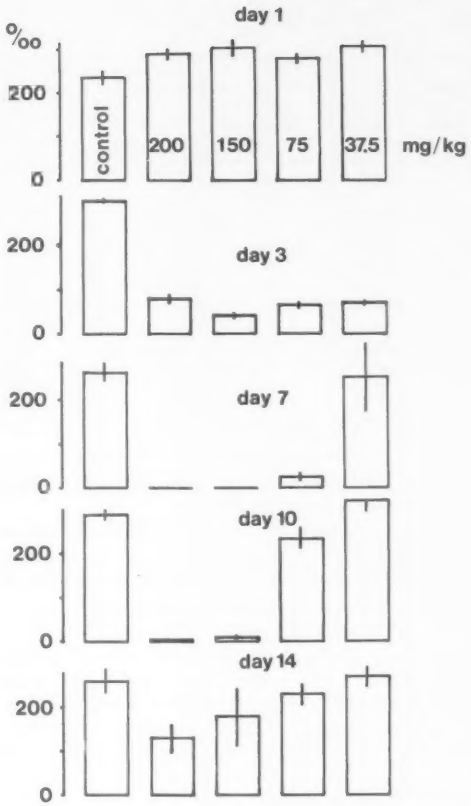


FIGURE 2.—Effect of a single dose of 5-FU ip on reticulocytosis after F-MuLV-P infection.

Effect of Single Doses of 5-FU on Hematopoietic Stem Cells

The effect of a 100-mg/kg ip dose of 5-FU was studied in uninfected and F-MuLV-P-infected mice (table 1). As seen from the same numbers of CFU-E with or without addition of Ep, the erythroid system in the bone marrow and spleen was completely Ep-independent in the F-MuLV-P-infected groups that were not given 5-FU (this was also the case in all other experiments and will not be mentioned further). After treatment, CFU-E were completely eliminated from the femur and the spleen by Day 1 in virus-infected mice and by Day 3 in both groups. The CFU-E seemed to be more sensitive in virus-infected mice. By Day 5 the erythroid regeneration had started in the femur and the spleen of uninfected and virus-infected mice. In the femur of these mice, no colonies were seen without addition of Ep; in

TABLE 1.—Effect of 100 mg/kg of 5-FU ip on CFU-C and CFU-E in femur and spleen of uninfected mice and those infected 12 days previously with F-MuLV-P

Days after 5-FU treatment	Study group	Bone marrow				Spleen			
		No. of cells/femur ($\times 10^6$)	No. of CFU-C/femur	No. of CFU-E/femur		Weight (mg)	No. of CFU-C/spleen	No. of CFU-E/spleen	
				−Ep	+Ep			−Ep	+Ep
1	Uninfected	15.4	10,270	—	17,400 ± 520	109 ± 7	1230	—	29,340 ± 1090
	+ 5-FU	10.7	200	—	750 ± 200	100 ± 5	100	—	0
	F-MuLV-P	12.5	8910	5625 ± 370	5125 ± 310	2240 ± 240	21,700	4.8×10^6	5.4×10^6
	+ 5-FU	9.5	0	0	0	830 ± 135	0	0	0
3	Uninfected	14.3	17,920	—	13,440 ± 280	94 ± 19	3050	—	29,300 ± 1000
	+ 5-FU	3.9	810	—	0	98 ± 10	80	—	0
	F-MuLV-P	10.2	13,700	820 ± 290	1120 ± 200	1285 ± 180	122,980	3.3×10^6	3.1×10^6
	+ 5-FU	3.5	70	0	0	181 ± 27	0	0	0
5	Uninfected	16.8	18,820	—	13,270 ± 1000	121 ± 9	—	—	20,670 ± 4600
	+ 5-FU	3.9	30	—	1290 ± 120	101 ± 22	710	—	12,110 ± 1640
	F-MuLV-P	11.3	—	3050 ± 380	2710 ± 320	1700 ± 420	69,070	4.7×10^6	4.7×10^6
	+ 5-FU	1.8	840	0	120 ± 90	143 ± 28	3450	17,120 ± 2310	15,690 ± 2730

the spleen there was no significant difference in the colony number with or without Ep.

In the next experiment, 37.5 and 75 mg/kg were used and the CFU-C and CFU-E of the femur were measured in uninfected and F-MuLV-P-infected mice (table 2). There was a dose-dependent reduction and regeneration of CFU-C and CFU-E. Normal CFU-E (those requiring the addition of Ep) were seen in F-MuLV-P-infected mice at Day 3 after the lower 5-FU dose and in very small numbers at Day 5 after the higher dose. Furthermore, the reduction of the CFU-C and CFU-E numbers was more pronounced in the virus-infected mice.

Multiple Doses of 5-FU

A series of experiments was then done with 18.75-, 37.5-, and 50-mg/kg doses of 5-FU and also with multiple doses of 5-FU given at 12- or 24-hr intervals. In all experiments, the parameters were studied as described before, but the results are presented in a shorter form (figs 3 and 4). In all experiments, the daily control for F-MuLV-P-infected mice (CFU-E per femur) varied to the same extent as in tables 1 and 2 and was set as 100% for each day. CFU-E numbers in 5-FU-treated mice were related to this daily control, and the colonies grown with and without Ep were given in percent. The two lower doses of 5-FU were followed by an overshoot in CFU-E per femur (fig 3). By Days 4–6, all groups had both Ep-dependent and Ep-independent CFU-E; the latter showed a relative increase with time. After two injections of 18.7 mg/kg at 24- or 12-hr intervals, the majority of CFU-E seen at Days 3 and 4 after treat-

TABLE 2.—Effect of 37.5 or 75 mg/kg of 5-FU ip on CFU-C and CFU-E in femur and spleen of uninfected mice and those infected 12 days previously with F-MuLV-P

Days after 5-FU treatment	5-FU dose (mg/kg)	Uninfected mice			F-MuLV-P-infected mice			
		No. of cells/femur ($\times 10^6$)	No. of CFU-C	No. of CFU-E	No. of cells/femur ($\times 10^6$)	No. of CFU-C	No. of CFU-E	
							−Ep	+Ep
3	Untreated	15.2	30,600*	10,490 ± 380	11.3	18,570	11,640 ± 380	10,850 ± 660
	75	5.4	2160	5350 ± 240	2.6	360	0	0
	37.5	7.9	3160	13,190 ± 450	5.3	0	640 ± 90	1960 ± 200
5	Untreated	16.9	24,790	21,460 ± 880	11.2	14,670	5890 ± 420	5710 ± 210
	75	5.8	20,300†	7420 ± 280	3.1	4960	300 ± 30	510 ± 60
	37.5	12.9	35,130†	13,670 ± 490	7.9	0	12,640 ± 670	13,035 ± 390
7	Untreated	16.0	23,840	22,240 ± 470	11.5	15,840	20,590 ± 390	20,120 ± 600
	75	10.3	12,360	10,400 ± 510	6.8	10,620	5640 ± 540	6120 ± 460
	37.5	11.5	22,340	8510 ± 580	9.2	7270	12,790 ± 370	12,510 ± 330
10	Untreated	13.0	14,470	17,290 ± 605	12.3	12,710	3570 ± 240	3320 ± 230
	75	16.4	18,040	13,450 ± 670	8.8	6930	8540 ± 600	8180 ± 430
	37.5	16.8	16,850	14,280 ± 500	9.8	7690	4900 ± 250	5000 ± 400

*SEM of CFU-C determinations ± 8%.
†Small colonies.

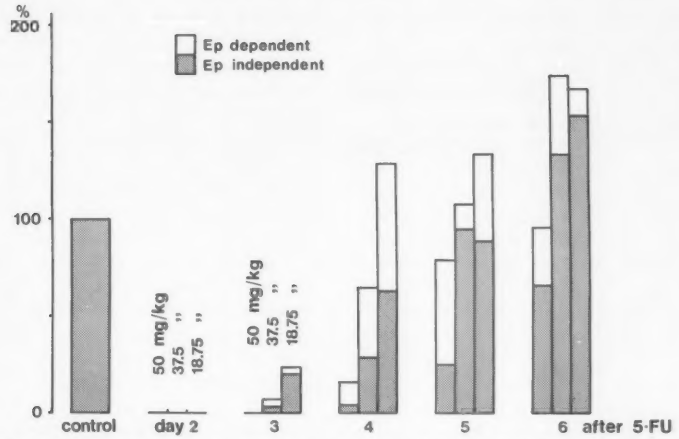


FIGURE 3.—Effect of 5-FU treatment on CFU-E per femur in mice infected with F-MuLV-P 12 days previously. Untreated control groups were studied at each experimental day and the number of CFU-E per femur was set as 100%; all colony growth in these F-MuLV-P-infected mice was Ep-independent.

ment were Ep-dependent (fig 4). But later, at Days 5–7 after two or even three injections of 18.7 mg/kg of 5-FU at 12-hr intervals, all CFU-E growth was Ep-independent again (data not shown).

Effect of 5-FU on Normal and F-MuLV-P-Transformed Erythropoiesis

In some of the experiments described before, the depression of the CFU-E growth was more pronounced in F-MuLV-P-infected mice than in uninfected mice after treatment with 5-FU, marginally so at Day 5 after 100 mg/kg (table 1) and clearly so at Days 3 and 5 after 37.5 and 75 mg/kg (table 2). In a further experiment, single doses of 18.75 and 37.5 mg/kg were studied in uninfected and F-MuLV-P-infected mice (fig 5). The results confirm both observations made in the previous experiments: the induction of a substantial number of Ep-dependent CFU-E and the more pronounced effect of 5-FU in F-MuLV-P-infected mice. After a dose of 37.5 mg/kg, the CFU-E number in uninfected mice did not fall below 10% at Days 1 and 2 and showed an overshoot at Days 3 and 4. CFU-E numbers in infected mice given 37.5 mg/kg were lower and no overshoot was seen, even at Day 4. After a dose of 18.75 mg/kg, the reduction in uninfected mice persisted until Day 4, with a minimum at Day 2 and no overshoot. In infected mice, lower numbers were seen at Days 1 and 2, and they were followed by an overshoot at Day 4.

In the final experiments, uninfected and F-MuLV-P-infected DBA/2 mice were treated with 18.75 or 37.5 mg/kg of 5-FU, and the CFU-S, CFU-C, and CFU-E

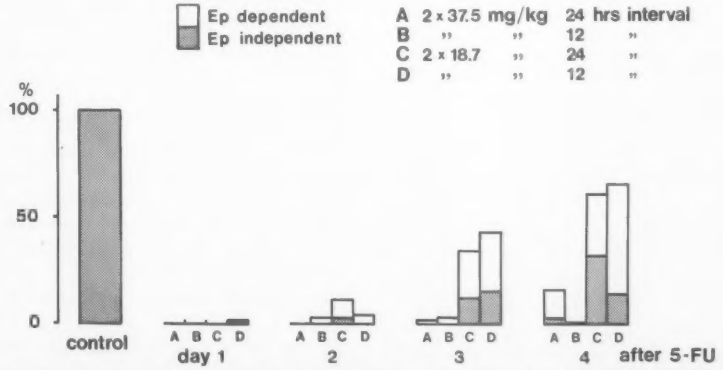


FIGURE 4.—Effect of 5-FU treatment on CFU-E growth per femur, with % calculated as per fig 3.

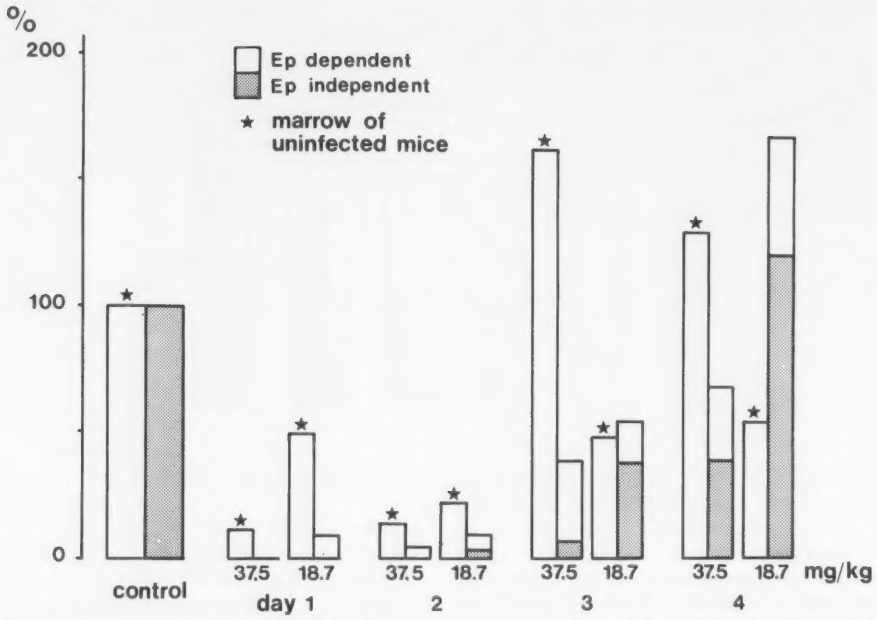


FIGURE 5.—Depression and regeneration of CFU-E numbers in the femur of uninfected (with stars) and F-MuLV-P-infected DBA/2 mice after treatment with 18.75 or 37.5 mg/kg of 5-FU. The % was calculated as per fig 3.

were studied at Days 1, 2, 4, and 7. The effect on the CFU-E compartment was as in the previous experiments, with an overshoot at Day 4 and > 50% Ep-dependent CFU-E after both doses; by Day 7 all CFU-E growth was Ep-independent again. The CFU-S and CFU-C data of Days 1 and 2 after 37.5 mg/kg are included in table 3, which shows lower values at Day 2 for all stem cell types from infected mice compared to values for uninfected controls.

DISCUSSION

Our results show that this viral leukemia can be temporarily suppressed by 5-FU and brought into a remission in the sense that Ep-dependent normal erythropoiesis is induced. This was also seen during regeneration after hydroxyurea treatment (6). Both drugs preferentially kill cells in S-phase, but the cytotoxicity of 5-FU in higher doses is not limited to this part of the cell cycle, as determined from many reports of the high kill of CFU-S in normal uninfected mice [(9,14–16), present study]. Such a treatment should be advantageous, since F-MuLV-P infection is thought to be restricted to cells in active turnover and there is strong evidence that the hematopoietic progenitor cells must be in cycle in order to be transformed by the virus (17–20).

TABLE 3.—Effect of a single dose of 37.5 mg/kg of 5-FU on hematopoietic stem cell regeneration in uninfected controls and mice infected 12 days previously with F-MuLV-P

	Study group	Regeneration after 5-FU*		No. of cells/femur
		Day 1	Day 2	
CFU-S	Uninfected	34	32	5400 ± 300
	F-MuLV-P	34	17	3500 ± 200
CFU-C	Uninfected	5	60	16,500
	F-MuLV-P	6	4	12,000
CFU-E	Uninfected	22	42	22,000 ± 400
	F-MuLV-P	8	0	500 + 300

*Values are % of values in untreated mice.

The results indicated that high doses of 5-FU are followed by a rather late regeneration of CFU-E, even later in infected mice than in uninfected mice. The CFU-E that were present at Days 5 and 7 after doses of 37.5, 75, and 100 mg/kg were all Ep-independent. Lower doses were followed by an earlier regeneration, and Ep dependency was seen, again followed by Ep independency at Day 5–7. These data suggest the following interpretation: 5-FU kills proliferating cells in a dose-dependent manner and regeneration starts from surviving pluripotent stem cells that were in G_0 and not infected. In the case of a high kill, regeneration is slow and consequently cells reaching the target cell level were infected completely. After a lower cell kill, regeneration is faster, comparable to the recovery after multiple doses of hydroxyurea, and consequently a first erythroid wave occurred before infection took place.

The second phenomenon observed concerns the longer depression of stem cells in virus-infected animals compared to uninfected mice. This agrees with a higher turnover of these cells in F-MuLV-P-infected mice. Data in the literature on CFU-S show a higher percentage in DNA synthesis, but a variation seems to exist (21,22). Our own unpublished data using hydroxyurea (2 hrs after 900 mg/kg) or the ^{3}H-thymidine suicide method showed kill rates of 5%–63% for CFU-S, compared to 10%–15% for controls. The corresponding values for CFU-E were 70% and 90% for infected and uninfected mice, respectively. 5-FU treatment has been suggested as a suitable tool for the study of cell turnover, exceeding the determination of the fraction of a cell population in S-phase (23), and the data presented seem to illustrate this possibility.

This paper finishes our series on the effect of cytotoxic drugs on hematopoietic stem cells in Friend leukemia. The series has included drugs with different modes of action, although they all led to a reduction in the spleen size: busulfan (4), with its major activity on low-cycling CFU-S; dactinomycin (4,5), with an almost selective effect on the erythroid cell system including CFU-E; carmustine (24), an alkylating agent; hydroxyurea (6); and now 5-FU. As discussed, only after hydroxyurea and 5-FU treatment could a short period be seen where most of the detectable CFU-E were normal again. This indicates that the F-MuLV-P-infected stem cells are rapidly cycling cells that can be eliminated, and that the regeneration of normal erythropoiesis starts from an uninfected precursor compartment. After some days, however, the virus causes a rapid transformation. The regeneration of Ep-independent erythropoiesis after dactinomycin treatment has been explained by the persistence of virus-infected precursors, most probably early erythroid-committed stem cells, BFU-E (5). Busulfan and carmustine, in this experimental system, are too toxic for the cell compartments that are responsible for the hematopoietic regeneration. Therefore, the erythroid system regenerated more slowly and was immediately Ep-independent after treatment, either due to the survival of some infected precursors or by reinfection.

In conclusion, this viral leukemia can be suppressed and temporarily reversed by drugs with a preferential action on proliferating cells; it cannot be cured by these, however, due to the persistence of the virus. Antiviral chemotherapy either alone or in combination with hydroxyurea was without success (25,26). In view of these results, we hope that the findings presented in this study contribute to the understanding of the hematopoietic compartments in an experimental leukemia. With respect to therapeutic success, it is obvious that chemotherapy of this virus-induced malignancy, even with the most effective drugs, has to be combined with other approaches, especially immunologic ones such as those presented for Friend leukemia by Schäfer et al (27) and in more recent studies (28).

REFERENCES

1. Liao S, and Axelrad AA. Erythropoietin-independent erythroid colony formation in vitro by hemopoietic cells of mice infected with Friend virus. Int J Cancer 15:467–482, 1975.
2. Horoszewicz JS, Leong SSS, and Carter WA. Friend leukemia: rapid development of erythropoietin-independent hematopoietic precursors. JNCI 54:265–267, 1975.
3. Opitz U, Seidel HJ, and Bertoncello I. Erythroid stem cells in Friend virus infected mice. J Cell Physiol 96:95–104, 1978.
4. Seidel HJ, and Opitz U. Effect of actinomycin D and busulfan on stem cells in normal and Friend virus infected mice. Blut 38:349–360, 1979.
5. Seidel HJ, Kreja L, Opitz U, et al. Normal and Friend virus specific cell compartments in Friend leukemia characterized by their sensitivity to actinomycin D in vivo. Blut 43:287–296, 1981.
6. Seidel HJ, and Opitz U. Hematopoietic stem cells in Friend murine leukemia virus-infected mice undergoing chemotherapy: remission and relapse of erythropoietin-independent erythropoiesis induced by hydroxyurea. JNCI 62:531–536, 1979.
7. Rosendaal M, Hodgson GS, and Bradley TR. Organization of haematopoietic stem cells: the generation-age hypothesis. Cell Tissue Kinet 12:17-30, 1979.
8. Madoc-Jones H, and Bruce WR. On the mechanism of the lethal action of 5-fluorouracil on mouse L-cells. Cancer Res 28:1976–1981, 1968.
9. Marsh JC. The effects of cancer chemotherapeutic agents on normal hematopoietic precursor cells: a review. Cancer Res 36:1853–1882, 1976.
10. Hodgson GS, and Bradley TR. Properties of haemopoietic stem cells surviving 5-fluorouracil treatment: evidence for a pre-CFU-S cell? Nature 281:381–383, 1979.
11. Wendling F, Tambourin PE, and Jullien P. Hematopoietic CFU in mice infected by the polycythemia inducing Friend virus. I. Number of CFU and differentiation pattern in the spleen colonies. Int J Cancer 9:554–566, 1972.
12. Till JE, and McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 14:213–222, 1961.
13. Iscove NN, and Sieber F. Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture. Exp Hematol 3:32–43, 1975.

14. Bruce WR, and Meeker BE. Comparison of the sensitivity of hematopoietic colony-forming cells in different proliferative states to 5-fluorouracil. JNCI 38:401–405, 1967.
15. Hršak I, and Pavičic S. Comparison of the effects of 5-fluorouracil and ftorafur on the haematopoiesis in mice. Biomedicine 21:164–167, 1974.
16. van Putten LM, Lelieveld P, and Kram-Idsenga LKJ. Cell-cycle specificity and therapeutic effectiveness of cytostatic agents. Cancer Chemother Rep 56:691–700, 1972.
17. Yoshikura H. Requirement of cellular DNA synthesis for the growth of Friend leukemia virus. Exp Cell Res 52:445–450, 1968.
18. Humphries EH, and Temin HM. Requirement for cell division for initiation of transcription of Rous sarcoma virus RNA. J Virol 14:531–546, 1974.
19. Kost TA, Koury MJ, Hankins WD, et al. Target cells for Friend virus-induced erythroid bursts in vitro. Cell 18:145–152, 1979.
20. Seidel HJ, and Kreja L. In vivo studies on hemopoietic stem cells and target cells for Friend virus infection in vitro. Exp Hematol 10:459–466, 1982.
21. Okunewick JP, and Phillips EL. Change in marrow and spleen CFU compartments following leukemia virus infection: comparison of Friend and Rauscher virus. Blood 47:885–892, 1973.
22. Tambourin PE, Wendling F, Jasmin C, et al. The physiopathology of Friend leukemia. Leuk Res 3:117–129, 1979.
23. Eaves AC, and Bruce WR. Altered sensitivity of hematopoietic stem cells to 5-fluorouracil (NSC-19893) following endotoxin (NSC-189681), cyclophosphamide (NSC-26271), or irradiation. Cancer Chemother Rep 58:813-820, 1974.
24. Seidel HJ, and Kreja L. Hematopoietic stem cells in Friend murine leukemia virus infected mice undergoing chemotherapy: failure to eradicate Ep-independent erythropoiesis by BCNU. Exp Hematol 10:151-160, 1982.
25. Opitz U, Seidel HJ, and Streissle G. Effects of ribavirin on the development of the Friend leukemia. Cancer Res Clin Oncol 98:41–49, 1980.
26. Seidel HJ, Opitz U, and Kreja L. Effects of tilorone on hemopoietic stem cells and on the development of Friend leukemia. Cancer Chemother Pharmacol 5:49–54, 1980.
27. Schäfer W, Schwarz H, Thiel HJ, et al. Properties of mouse leukemia viruses. XIII. Serum therapy of virus-induced murine leukemias. Virology 75:401–418, 1976.
28. Hunsmann G, Schneider J, and Schulz A. Immunoprevention of Friend virus-induced erythroleukemia by vaccination with viral envelope glycoprotein complexes. Virology 113:602–612, 1981.

Studies of the Pharmacology of *N*-Methylformamide in Mice[1,2]

Charles Brindley, Andreas Gescher,* Ernest S. Harpur, David Ross, John A. Slack, Michael D. Threadgill, and Helen Whitby[3,4]

When 400 mg/kg of ^{14}C-methyl-labeled *N*-methylformamide (NMF) was injected ip into mice, the curve for plasma concentration of radioactivity versus time was superimposable on the curve obtained by measuring unmetabolized NMF with gas-liquid chromatography during the first 24 hrs. Radioactivity in plasma was measurable for 8 days after NMF administration, but NMF was not measurable by gas chromatography beyond 24 hrs after administration. Radioactivity was eliminated from the plasma after 60 hrs, with an apparent half-life of 71.1 hrs. Of the radioactivity injected with NMF, 73.6% was recovered in the urine in 24 hrs; 26.4% of this was unchanged NMF. Three percent of the administered radioactivity was exhaled as $^{14}CO_2$ in 7 hrs at a constant rate of 0.007% per min. One urinary metabolite was a stable precursor of formaldehyde, which decomposed to formaldehyde only after alkaline hydrolysis and may well be *N*-(hydroxymethyl)-formamide. The areas under the plasma concentration versus time curve were estimated after ip, iv, and oral administration of NMF. The bioavailability of NMF was 1.01 after oral administration and 1.10 after ip administration. [Cancer Treat Rep 66:1957–1965, 1982]

The antitumor activity of *N*-methylformamide (NMF) ($OHC\text{-}NHCH_3$) against murine tumor models was first described by Clarke et al in 1953 (1). In a clinical trial, NMF was found to cause reversible liver damage in all seven patients in whom it was tested (2). We have recently found NMF to be active against the mouse ovarian reticulum cell sarcoma M5076 and the mouse TLX5 lymphoma (3). The animal tumors that are sensitive to NMF do not respond to treatment with other *N*-alkyl derivatives of formamide or with formamide itself.

The mechanism by which NMF exerts antitumor activity is unknown. We found NMF to be cytotoxic only at concentrations > 0.5 M against TLX5 lymphoma cells in an in vitro-in vivo bioassay (3). In this bioassay, *N*-ethylformamide (the ethyl analog of NMF), which is devoid of antitumor activity in vivo, exhibited cytotoxicity comparable to that of NMF.

Since NMF possesses antitumor activity against a considerable number of murine tumors and does not appear to be toxic to the bone marrow (4), it is a candidate for further clinical evaluation. To gain information that may facilitate the rational use of NMF in clinical trials, several aspects of its disposition in the mouse were studied. ^{14}C-Methyl-labeled NMF (^{14}C-NMF) was synthesized and the plasma disposition of radioactivity was compared with that of unlabeled NMF.

Renal excretion and metabolism to CO_2 are two obvious routes of elimination that a small molecular weight compound like NMF can undergo. Therefore, the appearance of radioactivity after administration of ^{14}C-NMF was studied in the urine and the breath. Special attention was given to identifying products of the oxidative metabolism of NMF in the urine, in view of our finding that formamide is a urinary metabolite (3).

The bioavailability of NMF after ip or oral administration was also investigated because in the antitumor tests, NMF was administered ip, whereas in the clinic, NMF may be given orally as well as iv or im. Finally, the influence of the presence of the TLX5 lymphoma on the plasma disposition of NMF was investigated.

MATERIALS AND METHODS

NMF and formamide were purchased from Aldrich Chemicals (UK); *N*-(hydroxymethyl)-formamide was synthesized by Mr. E. N. Gate in our laboratories ac-

[1]Received Mar 30, 1982; revised July 23, 1982; accepted Aug 16, 1982.
[2]Supported by grants from the Medical Research Council and the Cancer Research Campaign of Great Britain.
[3]CRC Experimental Chemotherapy Research Group, Department of Pharmacy, University of Aston, Birmingham, England.
[4]We thank Dr. J. A. Hickman for his interest in this work and Karen Ensor for her help with the chromatographic determination.
**Reprint requests to:* Dr. A. Gescher, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, England.

cording to a published method (5). ^{14}C-NMF was prepared by treatment of ^{14}C-methylamine hydrochloride (Radiochemical Centre, Amersham, UK) with ethyl formate and sodium carbonate; details of the synthetic procedure will be published elsewhere. Drugs were administered in solution in sterile saline. Male CBA CA mice (20–25 g) were used throughout this study. Approximately 10^5 TLX5 cells from routine passage as ascites were inoculated sc in the inguinal region of 20-g mice.

Measurement of NMF and total radioactivity in the plasma and urine.—The drug was injected into a tail vein iv, administered ip, or given by esophageal intubation. The doses administered were 400 mg/kg (equivalent to 1/2 × LD10 determined by ip administration) and 80 mg/kg. The amount of radioactivity injected into each mouse with the dose was either 5 or 15 μCi. While mice were under halothane anesthesia, blood samples (20 μl) were collected into heparinized capillary tubes from the tip of the tail for at least ten time intervals following drug administration. In a control experiment, blood was obtained without anesthetizing the animals, and plasma concentration versus time profiles were not different from profiles derived from anesthetized animals. Plasma was obtained by centrifugation, and samples were diluted with acetone and centrifuged again. Urine was collected from mice kept in metabolic cages (Jencons, UK). To minimize the risk of evaporative losses, the urinary collection funnels were washed with 10 ml of water after 2–6-hr intervals during the first 12 hrs and again 24, 48, and 72 hrs after drug administration. For the analysis by gas chromatography (GC), 10 μl of blood or 100 μl of diluted urine was further diluted with 50 or 200 μl of acetone (including internal standard). Radioactivity was counted after adding 10 μl of blood or 1 ml of diluted urine to 5 or 10 ml of NE260 Micellar scintillant (New England Nuclear, W Germany), in a Packard Tricarb 2660 scintillation counter using the external standardization mode. The centrifuged plasma or urine samples containing acetone with tetramethylurea as internal standard were analyzed by GC as described previously (3). The limit of detection for NMF was 3–5 μg/ml.

Breath analysis.—After iv administration of ^{14}C-NMF, the mice were placed in air-tight metabolic cages and the exhaled radioactivity was collected continually in a trapping fluid that was renewed at frequent intervals as previously described in reference 6. The trapping fluid did not contain measurable amounts of unmetabolized NMF as indicated by GC analysis, so that most of the exhaled radioactivity was considered to be in the form of $^{14}CO_2$.

Pharmacokinetic analysis.—The decline in plasma concentration of radioactivity with time beyond 24 hrs after drug administration was multiexponential. The decline of plasma concentration of radioactivity beyond 60 hrs after administration of ^{14}C-NMF was considered to represent the elimination of drug or metabolites, and in each animal the plasma concentration values obtained beyond 60 hrs after ^{14}C-NMF administration were subjected to a linear regression analysis that afforded a line (correlation coefficient > 0.98) from which a half-life was computed. The areas under the plasma concentration versus time curves (AUC) for unchanged NMF up to 24 hrs after NMF administration were estimated by the trapezoidal rule. AUC and half-life values as well as recoveries of NMF and radioactivity in the urine, as given in the Results section, are the mean ± SD from at least five animals. The bioavailability was calculated, using mean AUC data, from the ratio $\frac{\text{AUC ip or oral}}{\text{AUC iv}}$.

Analysis of formaldehyde precursors in the urine.—Urine samples were diluted and made alkaline by the addition of 1 N NaOH (to pH 12), and liberated formaldehyde was measured colorimetrically according to Nash (7).

RESULTS

NMF (400 mg/kg) was injected ip and its concentration in plasma was measured by GC and compared with the plasma disposition of radioactivity after administration of ^{14}C-NMF. Figure 1 shows that the plasma concentration versus time plot of NMF as measured by GC is very similar to that of total radioactivity during the first 24 hrs after drug administration. This suggests that unchanged NMF is the main constituent of the total radioactivity measured in the plasma. GC estimation of NMF in plasma samples collected beyond 24 hrs after NMF administration was unreliable since levels were generally near or below the detection limit of the assay. Radioactive species derived from ^{14}C-NMF were, however, measurable in the plasma for 8 days after drug administration (fig 2). The apparent concave downward curvature of the line up to 24 hrs on a semilog plot (fig 1) suggests that the disappearance of the drug from plasma does not obey first-order kinetics during this time. However, the disposition of radioactivity beyond 24 hrs can be described by a curve that appears to exhibit first-order kinetics with at least two exponential components (fig 2). Radioactivity was detectable in the plasma up to 192 hrs after administration of ^{14}C-NMF. The decline of plasma concentration of radioactivity with time between 60 and 192 hrs after drug administration is linear and may well represent the elimination of radioactivity, ie, of NMF and/or its metabolites. (Elimination rate constant $K_{el} = 0.0097 \pm 0.0012$ hr^{-1}; elimination half-life = 71.1 ± 8.8 hrs.)

A total of 73.6% ± 6.8% of the radioactivity was recovered in the urine within 24 hrs after ip administration of 400 mg/kg of ^{14}C-NMF. Figure 3 shows that

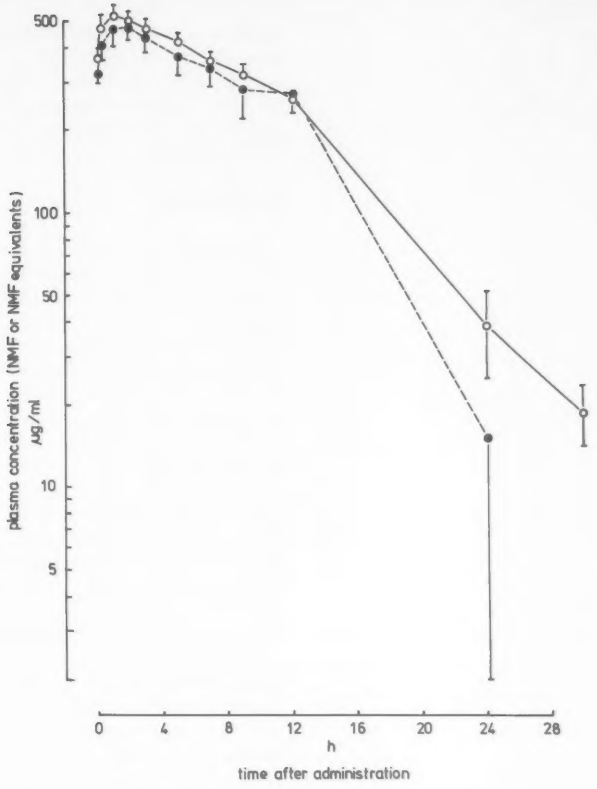


FIGURE 1.—Plasma disposition of NMF measured by GC (●) and of total radioactivity (○) after ip administration of 400 mg/kg of NMF or ^{14}C-NMF. Concentrations of radioactivity are expressed as NMF equivalents. Values are the mean ± SD of 6 experiments, except for the point without an error bar, which is the mean of 3 experiments.

26.4% ± 2.9% of the radioactivity in the urine was unchanged NMF and < 2% (not shown in fig 3) of the dose was eliminated as either formamide, the *N*-demethylated metabolite of NMF, or its *N*-hydroxymethyl precursor, *N*-(hydroxymethyl)-formamide ($OHCNHCH_2OH$); the latter also appears as formamide on GC determination. Some urinary metabolites (or a metabolite) of NMF were hydrolyzed to formaldehyde when urine samples were treated with NaOH, and the hydrolysate reacted with Nash reagent. In two experiments, 4.4 µmols of the metabolites (expressed as formaldehyde equivalents) were excreted with the urine in 36 hrs. *N*-(Hydroxymethyl)-formamide releases formaldehyde on alkaline hydrolysis, so the urinary formaldehyde precursor may well be this carbinolamine. Control animals and those that received *N*-ethylformamide did not excrete formaldehyde precursors sensitive to alkali.

Radioactivity exhaled with the breath as $^{14}CO_2$ after administration of 400 mg/kg of ^{14}C-NMF was collected for 7 hrs. Only 3% of the radioactivity injected was exhaled during this time, at a constant rate of 0.007%/min.

When NMF was administered iv or orally, the disappearance of drug from plasma with time did not substantially differ from the disappearance of NMF from the plasma after ip injection. In figures 4 and 5, graphs of NMF plasma concentrations plotted on a linear scale against time after administration of two iv (fig 4) and oral (fig 5) doses of NMF (80 and 400 mg/kg) reveal apparently linear sections, which indicate that zero-order kinetics might partially govern these plasma disposition profiles. The time required for plasma levels to decline from peak concentrations to 50% after oral administration of NMF (fig 5) was 9.5 hrs for the high dose and 8 hrs for the low dose.

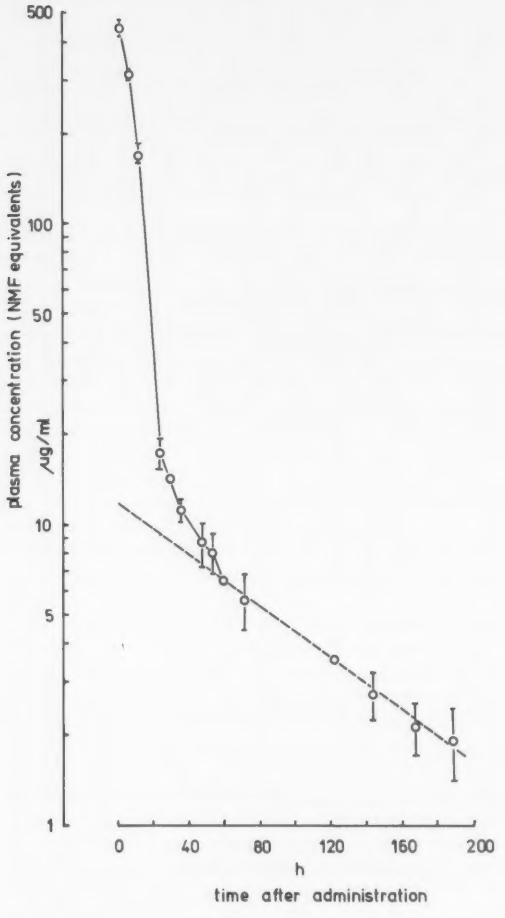


FIGURE 2.—Plasma disposition of radioactivity after ip administration of 400 mg/kg of ^{14}C-NMF. Values are the mean ± SD of 6 experiments; the values without error bars are the mean of 3 experiments. Dashed line indicates the line obtained by linear regression analysis of points beyond 60 hrs after drug administration.

AUC values were calculated up to 24 hrs after administration of 400 mg/kg of NMF and were 5235 ± 1270 $\mu g \cdot hr \cdot ml^{-1}$ after iv administration, 5842 ± 783 $\mu g \cdot hr \cdot ml^{-1}$ after ip administration, and 5360 ± 1117 $\mu g \cdot hr \cdot ml^{-1}$ after oral administration. Thus, the systemic availability of NMF was 1.01 after oral administration and 1.10 after ip injection.

Mice were inoculated with the TLX5 lymphoma, which is sensitive to NMF (3). Five days after tumor implantation (approximately half the lifespan of untreated tumor-bearing mice), a plasma concentration versus time profile was determined after ip injection of NMF in these mice. It was virtually identical with the profile seen in normal mice. The AUC calculated from the profile in six tumor-bearing mice was 6595 ± 564 $\mu g \cdot hr \cdot ml^{-1}$ and did not differ significantly from the value obtained in normal mice.

DISCUSSION

Radioactivity injected as a single dose of ^{14}C-NMF (400 mg/kg) was detected in plasma for 8 days after drug administration, and the plasma radioactivity concentration versus time curve (fig 2) shows that a drug disposition phase that can be described by first-order kinetics was not reached until 24 hrs after NMF administration. After 60 hrs, radioactivity was cleared from the plasma with an apparent half-life of 71.1 hrs.

The physicochemical nature of NMF and its potential metabolites has so far prevented the unequivocal identification of the radiolabeled species present in this late phase of NMF disposition; the high water:octanol partition coefficient of NMF and its likely metabolites (unpublished results) prevents efficient extraction into organic solvent and their high volatility renders freeze-drying of samples inappropriate.

Repeated daily administration of high doses of NMF was required for optimal antitumor activity in murine tumor models (3), and it is likely that to be effective in patients, NMF may have to be given in high doses on multidose schedules or by constant infusion. If the long elimination half-life of drug-derived species observed in mice (fig 2) is also found in man, then repeated dosing at frequent intervals will lead to drug accumulation. Whether accumulation of NMF or its metabolites is a prerequisite for its antitumor efficacy or the generation of its hepatotoxicity remains to be elucidated.

The plasma disposition profile of radioactivity during the first 24 hrs after drug administration is virtually superimposable on that of unlabeled NMF (fig 1), which shows that only a small amount of metabolite(s), if any, appears in the plasma during this time. However, metabolites were found in the urine (fig 3), which suggests that after their formation, the metabolites (whatever their chemical nature) are rapidly eliminated into the urine.

The plasma concentration versus time curves of NMF (as measured by GC) within 24 hrs after ip (fig 1), iv, and oral administration of 400 mg/kg exhibit a concave downward curvature when plotted semilogarithmically. This curvature was not seen in a preliminary study of NMF plasma levels (3) when blood was obtained by cardiac puncture. The mathematical treatment of these data, based on linear kinetics, might not be appropriate and the concept of plasma elimination rates and half-lives cannot be used to describe these curves. When plotted using Cartesian coordinates, concentration val-

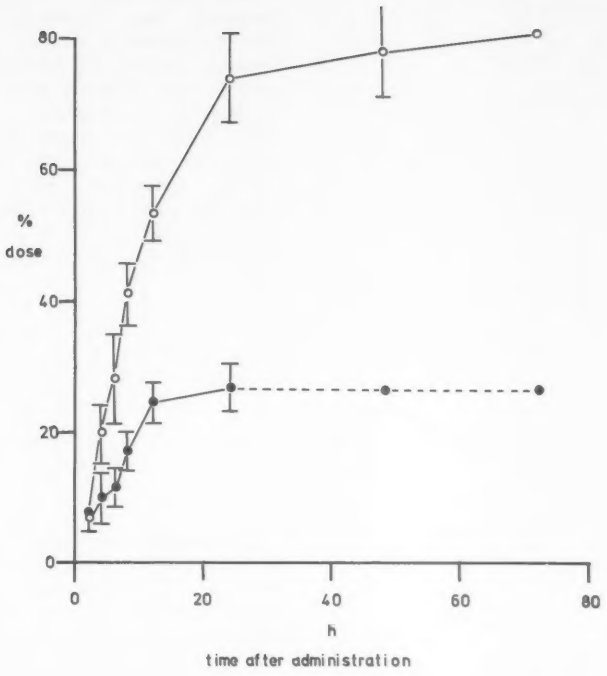


FIGURE 3.—Cumulative urinary excretion of NMF measured by GC (●) and of total radioactivity (○) after ip administration of 400 mg/kg of NMF or ^{14}C-NMF, expressed as % of dose administered. Values are the mean ± SD of 5 experiments. There were no measurable levels of unchanged NMF in the urine after 24 hrs.

ues during some periods of the disposition appear to fit a straight line (figs 4 and 5), which indicates that NMF might be eliminated at a fixed rate independent of its concentration in the plasma (zero-order kinetics). Nonlinear pharmacokinetics are often associated with the saturation of the pathways by which a drug is distributed in or eliminated from the body, eg, uptake into tissues or hepatic metabolism (8). The data in figs 4 and 5 suggest either that both doses are above the saturable point or that the removal process is zero-order regardless of the dose in the first 24 hrs. When plasma concentration values obtained within 24 hrs after administration of 80 and 400 mg/kg of NMF are divided by the dose and the ratios are plotted against time (fig 6), the resulting curves, although not superimposed, are not conspicuously different from each other [nonlinear kinetics must be suspected if the curves are not superimposable (8)]. This does not facilitate a conclusion as to whether or not the plasma disposition of NMF is governed by nonlinear kinetics. No further attempt has yet been made to determine which model best describes the plasma disposition of NMF. Further studies are required to unambiguously establish nonlinearities in the pharmacokinetics of NMF. To define the kinetic model for the disposition is important because it may allow the prediction of drug levels in the body and, as a consequence, the prediction of therapeutic or unwanted drug effects.

Most of the radioactivity injected with NMF was excreted into the urine within 24 hrs (fig 3). Only 26.4% of the dose appeared in the urine as unchanged NMF and < 2% as formamide [or *N*-(hydroxymethyl)-formamide]. To identify the urinary metabolite(s) of NMF is relevant in view of the suggestion that NMF may require metabolic activation to exert its antineoplastic activity (3). Since the metabolite(s) were cleared from

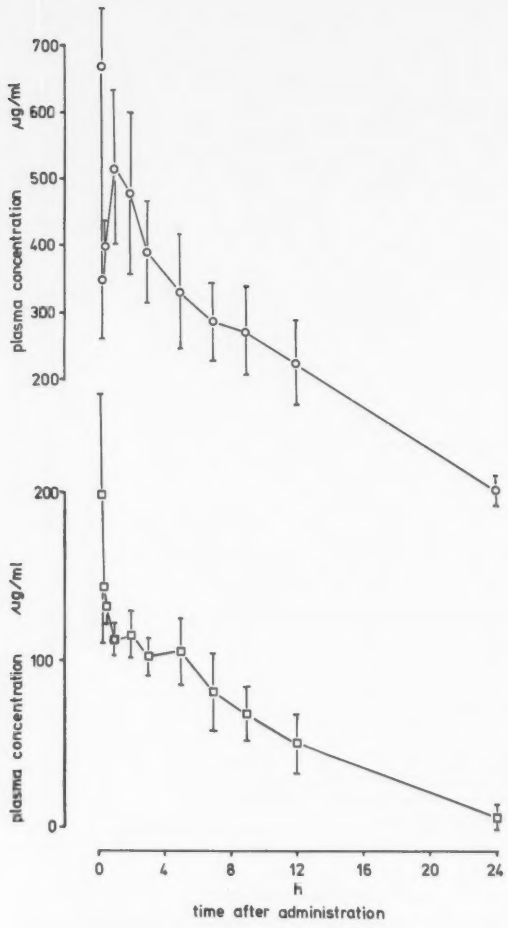
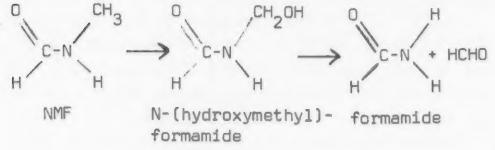


FIGURE 4.—Plasma disposition of NMF after iv administration of 80 mg/kg (□) or 400 mg/kg (○) of NMF. Values are the mean ± SD of at least 5 mice.

the blood rapidly, it is possible that they are very hydrophilic conjugates. Indeed, on alkaline hydrolysis, urine of NMF-treated mice contained a large amount of thiol compounds, suggesting the presence of metabolites with thioether structure, such as *N*-acetylcysteine derivatives (A. Gescher, unpublished observation). One metabolite was characterized as a stable precursor of formaldehyde, presumably *N*-(hydroxymethyl)-formamide. Preliminary unpublished results show that this species does not possess the antitumor efficacy of NMF and does therefore not appear to be an active metabolite. The evidence for the presence in urine of *N*-(hydroxymethyl)-formamide as a metabolite of NMF suggests the following metabolic scheme:



It is puzzling that radioactivity excreted with the breath as $^{14}CO_2$, the final oxidation product of the NMF methyl moiety, was exhaled at a constant rate of 0.007%/min during 7 hrs after drug administration. The $^{14}CO_2$ exhalation pattern observed with ^{14}C-NMF contrasts with the profiles reported after administration of other drugs that undergo metabolic *N*-demethylation, eg, hexamethylmelamine, aminopyrine, and caffeine. In these cases, the $^{14}CO_2$ exhalation profiles reflect the plasma disposition of drug-derived *N*-methyl moieties (6). It is possible that in the case of NMF, the *N*-hydroxymethyl metabolite is so stable that further metabolism (eg, cleavage of the N-C bond to form formaldehyde), since it does not occur spontaneously, is catalyzed by an enzyme that is saturated on administration of large doses of NMF. This might lead to a slow zero-order formation of CO_2, probably circumventing free formaldehyde in the metabolic process, at a rate that is independent of plasma NMF concentration and that in turn determines the rate at which $^{14}CO_2$ appears with the breath.

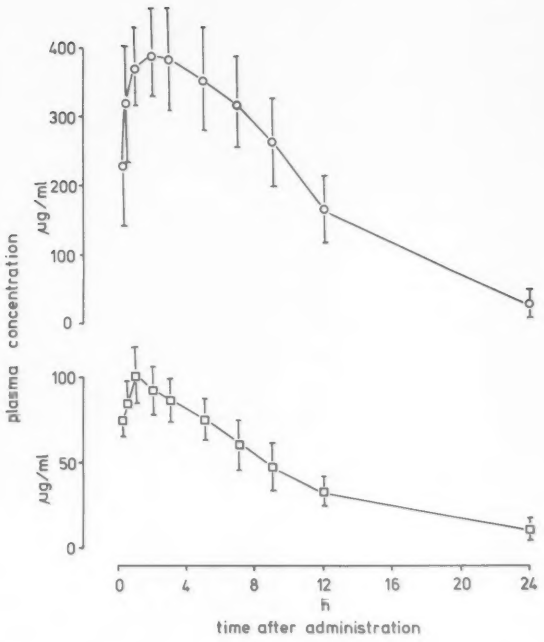


FIGURE 5.—Plasma disposition of NMF after oral administration of 80 mg/kg (□) or 400 mg/kg (○) of NMF. Values are the mean ± SD of 4–8 mice.

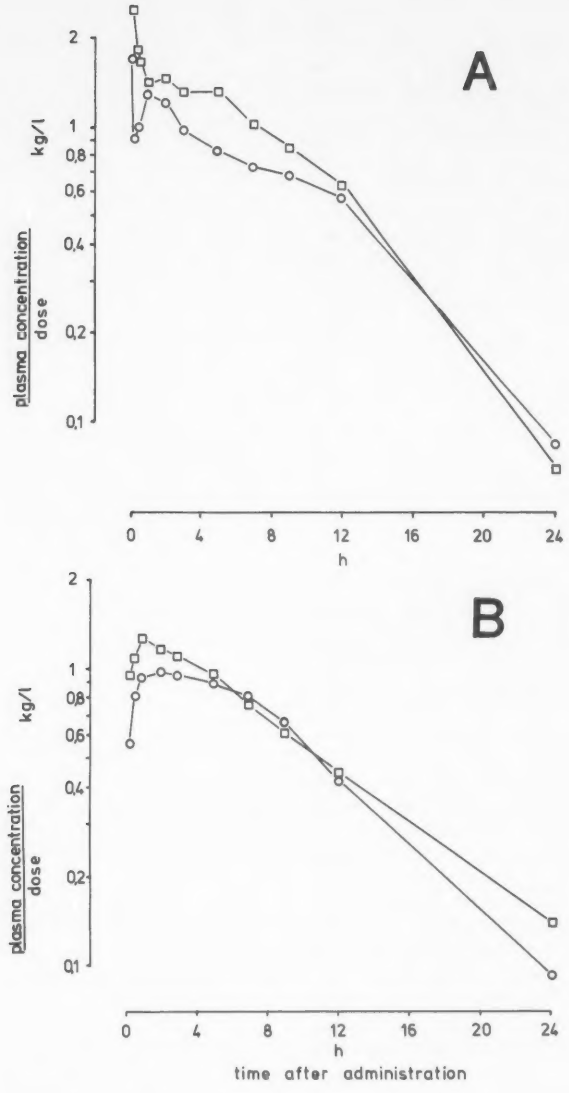


FIGURE 6.—Plots of NMF plasma concentrations divided by the dose against time after iv (A) or oral (B) administration of either 80 mg/kg (□) or 400 mg/kg (○) of NMF. Values are identical with the mean values shown in figs 4 and 5.

REFERENCES

1. CLARKE CA, PHILIPS FS, STERNBERG SS, ET AL. Effects of *N*-methylformamide and related compounds in sarcoma 180. Proc Soc Exp Biol Med 84:203–207, 1953.
2. MYERS WPL, KARNOFSKY D, and BURCHENAL JH. The hepatotoxic action of *N*-methylformamide in man. Cancer 9:949–955, 1956.
3. GESCHER A, GIBSON NW, HICKMAN JA, ET AL. *N*-Methylformamide: antitumour activity and metabolism in mice. Br J Cancer 45:843–850, 1982.
4. NEWMAN KN, MEEKS RG, and FRICK S. Phase 1 report of task 1 preclinical intravenous toxicity study of *N*-methylformamide (NSC 3051) in CDF_1 mice and beagle dogs. Birmingham, AL, Southern Research Institute, 1981.
5. EINHORN A. Ueber die N-Methylolverbindungen der Säureamide. Justus Liebig's Ann Chem 343:207–310, 1905.
6. GESCHER A, and RAYMONT C. Studies on the metabolism of *N*-methyl containing anti-tumour agents. $^{14}CO_2$ breath analysis after administration of ^{14}C-labelled *N*-methyl drugs, formaldehyde and formate in mice. Biochem Pharmacol 30:1245–1251, 1981.
7. NASH T. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. J Biol Chem 56:412–416, 1953.
8. WAGNER JG. A modern view of pharmacokinetics. J Pharmacokinet Biopharm 1:363–401, 1973.

Massive Vincristine Overdose: Failure of Leucovorin To Reduce Toxicity[1]

Lambert L. Thomas,[2,*] Paul C. Braat,[2] Reinier Somers,[3] and Robert Goudsmit[2]

The case history of a 24-year-old male with the nodular sclerosing form of Hodgkin's disease (stage II_E) who accidentally received an overdose of vincristine (25 mg, 0.39 mg/kg) is reported. To date, ten patients given a vincristine overdose have been reported. Two of those patients were treated with leucovorin. One patient died after 68 hours. In the second patient, the time to onset and the duration of symptoms were shortened. In an attempt to reduce the duration of toxic effects in our patient, leucovorin was administered 24 hours after the overdose. In agreement with the earlier observation, the time to onset of symptoms was short, but in contrast we did not find a more rapid recovery from the serious side effects. Although the patient might have died without leucovorin administration, we believe its beneficial effect remains to be established. [Cancer Treat Rep 66:1967–1969, 1982]

CASE REPORT

A few weeks before admission, a 24-year-old male reported a sharp pain in the right hemithorax, radiating to neck and shoulders and aggravated by respiratory movements. His temperature varied periodically between 37.5°C and 38.5°C.

Physical examination disclosed a dull percussion sound at the right sternal border. No other abnormalities were found.

Laboratory investigations showed an erythrocyte sedimentation rate of 40 mm, a leukocyte count of 17.8 $\times$ 10^9/L with a normal differential count, and a platelet count of 394 $\times$ 10^9/L. Transaminases and other liver enzymes were normal.

An X-ray of the chest showed a large, round shadow in the middle and upper field of the right lung. The shadow had a pyramid-shaped extension in the right upper lobe, with its base on the anterior thoracic wall.

A transthoracic puncture was performed. Cytologic examination of the aspirate showed granulocytes, eosinophils, and numerous Reed-Sternberg giant cells. A supraclavicular lymph node, appearing shortly afterward, enabled the histologic diagnosis of nodular sclerosing type Hodgkin's disease. Since further staging procedures showed no abnormalities, the Hodgkin's disease was classified as stage II_E.

A regimen of three MOPP courses (mechlorethamine, vincristine, procarbazine, and prednisone) followed by mantle field irradiation and completed by three MOPP courses was planned.

By accident, 25 mg of vincristine in 250 ml of supplied diluent was administered in the first treatment regimen. This error was not discovered until the next day. As an attempted prophylactic measure, 12 mg of leucovorin was administered im four times daily for 5 days, starting 24 hours after the vincristine overdose.

The following sequence of events occurred after the overdose (the time course of the development and resolution of symptoms, respectively, is given parenthetically). The patient had nausea, vomiting, and diarrhea (5 hours–4 days). He developed dyspnea, presumably caused by a discrete alveolar-capillary block: pH = 7.50, pCO_2 = 28 mm Hg, pO_2 = 67 mm Hg (10 hours–4 days). There was a short hypotensive period: Riva-Rocci reading = 95/70 and pulse frequency = 140/minute (10 hours–24 hours). Bone marrow depression was present from Day 4 on. The hematocrit, leukocyte count, and platelet count declined to 0.31 (Day 9), 1.7 $\times$ 10^9/L (Day 8), and 36 $\times$ 10^9/L (Day 6), respectively. On Day 8, segmented leukocytes were virtually absent in the peripheral blood. After a rapid rise, the values were normalized or markedly increased to 0.38, 34.3 $\times$ 10^9/L, and 790 $\times$ 10^9/L, respectively, on Day 16. In a few weeks, all values were within normal ranges.

The syndrome of inappropriate secretion of antidiuretic hormone (ADH) developed, which promptly responded to fluid restriction (Day 6–Day 10). A urine

[1]Received Dec 4, 1981; revised Aug 3, 1982; accepted Aug 19, 1982.

[2]Department of Hematology (*L. L. Thomas* and *R. Goudsmit*) and Department of Pulmonology (*P. C. Braat*), University Hospital, Wilhelmina Gasthuis, University of Amsterdam, The Netherlands.

[3]Antonie van Leeuwenhoek Hospital, Amsterdam, The Netherlands.

**Reprint requests to:* Dr. Lambert L. Thomas, Department of Hematology, University Hospital, Wilhelmina Gasthuis, University of Amsterdam, The Netherlands.

sample collected after the nadir of the syndrome showed normal levels of ADH. Parenchymal liver damage was maximal on Day 3, as reflected by elevated SGOT, SGPT, and LDH levels of 128, 208, and 2072 IU/ml, respectively (Day 3–Day 10). Substantial hair loss was noted from Day 12 on. Signs of cerebral dysfunction, eg, anxiety, depression, agitation, and hallucinations, developed after 4 days and gradually declined over several weeks.

The neurotoxicity of the overdose was severe, with paresthesias (Day 2–Day 80) and generalized pain in muscles, jaws, and abdomen (Day 3–Day 8). A complete paralytic ileus developed, necessitating parenteral feeding (Day 5–Day 16). Functional impairment of cranial nerves V, VI, and VIII was noted (Day 4–Day 9). Muscular weakness and loss of tendon reflexes were observed for a longer period (Day 4–Day 80). Bladder atony led to intermittent catheterization (Day 7–Day 16). An electroencephalogram in the acute phase showed no localizing abnormalities but was consistent with diffuse cerebropathy caused by the intoxication.

An impressive tumor regression was observed. The 234-cm^3 mass had virtually dissipated, leaving some ring shadows. A second cytostatic course was given, but without vincristine. No adverse effects were noted. An electromyogram made shortly before discharge showed no remaining signs of polyneuropathy. Six months after the vincristine overdose, neurologic examination still showed a slight paresis of both feet and atrophy of the muscles of both hands. Deep tendon reflexes were absent.

To date, 3 years after diagnosis, there is no evidence of relapse of the patient's Hodgkin's disease.

DISCUSSION

The signs and symptoms of vincristine overdose described in this patient have all been reported previously (1–7). The overdoses have varied from 0.12 to 0.60 mg/kg. Even without an overdose, similar vincristine toxic effects such as peripheral and autonomic neuropathy, cranial nerve palsies, mental changes, and inappropriate ADH secretion are well-known (8). However, the sequelae of a massive overdose are much more serious. Four of the ten reported patients died, the deaths being directly related to vincristine toxicity (1,7). Two patients died of hemorrhagic complications due to bone marrow depression and one died of the complications of a paralytic ileus. The fourth patient died of a disseminated *Candida* infection on Day 24 when he was still profoundly leukopenic.

Maximal supportive care such as prophylaxis against seizures, assisted ventilation when required, blood component therapy during bone marrow depression, and treatment of inappropriate ADH secretion is considered crucial. A patient supported through the critical period may regain normal neurologic function as did our patient (1,6,7), although this may not be the rule (2,4).

Two patients reported were treated with leucovorin, based on the observation of a protective effect of leucovorin in mice given a lethal dose of vincristine (9). One patient died after 68 hours (1); the second recovered completely (6).

Major seizures due to vincristine overdose have been reported (1). Clinical evidence for an exacerbation of epilepsy after administration of folic acid, a structurally very similar molecule, is conflicting in the literature (10), but since our patient had no history of epilepsy we felt justified in administering leucovorin. Grush and Morgan (6) postulated the time to onset and the duration of symptoms to be shorter than those reported in previous publications (1–5,7), as shown in table 1. In agreement with Grush and Morgan, we noted a rather short time to onset of symptoms. However, in contrast, we did not find a more rapid recovery from the serious side effects (table 1).

Although the patient might have died without leucovorin administration, we believe its beneficial effects in vincristine overdose remain to be established.

TABLE 1 —Duration of toxicity in days following vincristine overdose

Toxic effect	Without leucovorin, refs 1–5	With leucovorin	
		Ref 6*	Present study†
Nausea/vomiting	1–7	1–2	1–4
Neuropathies	3–30	1–29	3–9
Inappropriate ADH	3–16	6–8	6–10
Paresthesias	7–28	7–18	2–80
Bone marrow depression	7–21	5–9	4–16
Gastrointestinal effects	9–21	3–9	3–16
Myopathies	14–60	6–15	4–80
Cerebral dysfunction	14–50	7–8	4–25

*Starting 48 hrs after vincristine overdose, 15 mg of leucovorin was given 8 times daily for 3 days.

†Starting 24 hrs after vincristine overdose, 12 mg of leucovorin was given 4 times daily for 5 days.

REFERENCES

1. KAUFMAN IA, KUNG FH, KOENIG HM, ET AL. Overdosage with vincristine. J Pediatr 89(4):671–674, 1976.
2. BERENSON MP. Recovery after inadvertent massive overdosage of vincristine (NSC-67574). Cancer Chemother Rep Part 1, 55(5):525–526, 1971.
3. FOOD AND DRUG ADMINISTRATION. Reports of suspected adverse reactions to drugs. 1969(3):9.
4. CASTEELS-VAN DAELE M, BEIRINCKX J, and BAINES P. Overdosage with vincristine. J Pediatr 6:1042–1043, 1977.
5. SUSKIND RM, BRUSILOW SW, and ZEHR J. Syndrome of inappropriate secretion of antidiuretic hormone produced by vincristine toxicity (with bioassay of ADH level). J Pediatr 81(1):90–92, 1972.
6. GRUSH OC, and MORGAN SK. Folinic acid rescue for vincristine toxicity. Clin Toxicol 14(1):71–78, 1979.
7. TOGHILL PJ, and BURKE JD. Death from paralytic ileus following vincristine therapy. Postgrad Med 46:330–331, 1970.
8. ROSENTHAL S, and KAUFMAN S. Vincristine neurotoxicity. Ann Intern Med 6:733–737, 1974.
9. JOHNSON IS, ARMSTRONG JG, GORMAN M, ET AL. The vinca alkaloids. A new class of oncolytic agents. Cancer Res 23:1390–1427, 1963.
10. REYNOLDS EH. Neurological aspects of folate and vitamin B_{12} metabolism. *In* Clinics in Hematology (Hoffbrand AV, ed). Philadelphia, W. B. Saunders, 1976, vol 5, No. 3, p 671.

Combination Chemotherapy for Multiple Myeloma With Melphalan, Prednisone, Cyclophosphamide, Vincristine, and Carmustine (BCNU) (M-2 Protocol)[1]

Umberto Tirelli,[2,*] Diana Crivellari,[2] Antonino Carbone,[3] Andrea Veronesi,[2] Enzo Galligioni,[2] Mauro G. Trovò,[2] Salvatore Tumolo,[2] and Eligio Grigoletto[2,4]

From January 1975 to May 1981, 25 consecutive patients with multiple myeloma (MM) were entered in a prospective study and treated with the M-2 protocol (melphalan, prednisone, cyclophosphamide, vincristine, and carmustine [BCNU]). The Karnofsky performance status was < 70 in 62% of the patients. Nineteen patients were classified as being in stage III, three were in stage II, and three were in stage I. All patients had symptomatic and previously untreated MM. In 17 of 21 (80.9%) evaluable patients, an objective response according to Myeloma Task Force criteria was obtained. There was no difference in response rate among patients in the various stages of the disease or according to types of proteins secreted. The median time to obtain an objective response was 2 months and the median duration of the remission is 18+ months. The actuarial median survival was 42 months. In the first 15 months of follow-up, 20% of the patients died. Toxicity was not negligible and was mainly hematologic. One treatment-related death occurred. Our study confirms the efficacy of the M-2 protocol in MM and supports the data reported by the Memorial Hospital group. [Cancer Treat Rep 66:1971–1973, 1982]

Memorial Hospital investigators claimed that the M-2 protocol (melphalan, prednisone, cyclophosphamide, vincristine, and carmustine [BCNU]) significantly improved the median survival of 46 previously untreated patients with multiple myeloma (MM) in comparison with historical controls (50 versus 22 months) (1). The same authors reported an objective remission rate of 77% according to Myeloma Task Force criteria (2). Surprisingly, few other groups have reported their experience with the M-2 protocol (2). Miller and Perrotta (3) obtained a 71% objective remission rate and a median survival of 21 months in 28 previously untreated patients with MM. Unfortunately, these results were reported in abstract form and no information regarding response criteria and toxicity was provided. Buonanno et al (4) observed three objective responses among nine evaluable patients treated with the M-2 protocol. However, the number of patients, all pretreated, was too small to draw any conclusion. Case (5), one of the coauthors of the two reports of the M-2 protocol at Memorial Hospital (1,6), published in letter form his own results in 20 patients treated between 1976 and 1979 with the M-2 protocol at Maine Medical Center. All patients responded and are alive except two who died from unrelated illness. Most recently, Paccagnella et al (7) reported in abstract form a 78% objective response rate and a median survival of 51 months in 56 previously untreated patients. These results in the largest series reported so far seem to confirm the Memorial Hospital data. However, the relative scarcity of information regarding the activity of the M-2 protocol and the debate about the real impact of the M-2 protocol on survival of patients with MM (5,8) prompted us to report our results with this regimen in a group of patients admitted to a large general hospital.

MATERIALS AND METHODS

From January 1975 to May 1981, 25 consecutive patients with previously untreated and symptomatic MM entered this prospective study. Staging procedures included routine blood analysis, serum and urine immu-

[1]Received Jan 6, 1982; revised June 21, 1982; accepted June 29, 1982.

[2]Division of Radiotherapy and Medical Oncology, General Hospital, Pordenone, Italy.

[3]Department of Pathology, Istituto Scientifico per lo Studio e la Cura dei Tumori, Genova, Italy.

[4]We thank Drs. Capra and Bassignano, Department of Physics, for statistical analysis.

Reprint requests to: Umberto Tirelli, MD, Division of Radiotherapy and Medical Oncology, General Hospital, 33170 Pordenone, Italy.

noelectrophoresis, bone marrow aspiration, and roentgenographic skeletal survey. Clinical staging according to Durie and Salmon (9) was applied to all patients. Combination chemotherapy with the M-2 protocol was given every 5 weeks as follows: 0.5 mg/kg of BCNU iv on Day 1, 10 mg/kg of cyclophosphamide iv on Day 1, 0.25 mg/kg of melphalan orally on Days 1–4, 1 mg/kg of prednisone for 7 days and then tapered until Day 21, and 0.03 mg/kg of vincristine iv on Day 21 (6). If an objective response occurred, chemotherapy was continued until 12 cycles had been completed.

Dosage of the drugs was reduced when leukothrombocytopenia was present and/or in critically ill patients. Following the start of chemotherapy, paraprotein levels and bone marrow plasma cell rates were serially evaluated, usually every 2 months for the first 4 months and then every 3–4 months. Clinical response was evaluated in accordance with Myeloma Task Force criteria (2). The duration of response started when the paraprotein level fell to < 50% of the control value and was calculated until the paraprotein level climbed to twice its lowest level during remission. Patients were evaluated for response when at least two cycles of the M-2 protocol had been given. Survival was calculated from the start of combination chemotherapy by means of the life-table method (10). Responses and survival were calculated as of May 1982.

RESULTS

As of May 1981, 25 consecutive patients had entered this study. Thirteen were males, 12 were females, and the median age was 65 years (range, 37–79). The Karnofsky performance score was < 70 in 62% of the patients. Nineteen patients were classified as being in stage III, three were in stage II, and three were in stage I. However, all patients had symptomatic disease. Twelve patients had IgG myeloma (four κ type and eight λ type), four had IgA myeloma (three κ type and one λ type), one had IgD myeloma of the λ type, and eight had micromolecular myeloma with measurable κ (four patients) and λ (four patients) light chains.

The median follow-up time was 23 months (range, 3–75). Four patients were not evaluable for response because they did not receive at least two cycles of the M-2 protocol. In 17 of the 21 (80.9%) evaluable patients, an objective response was achieved. Patients with stage III or stage I–II disease responded equally well (80% versus 81.2%, respectively). There was no difference in response with respect to the type of myeloma proteins secreted. The median time from the first cycle of M-2 to an objective response was 2 months. The median duration of remission is 18+ months (range, 3–69+).

All 25 patients are evaluable for survival. The actuarial 4-year survival curve is shown in figure 1. The median duration of survival was 42 months. In the first 15 months of follow-up, 20% of the patients died.

Toxicity was not negligible. During the entire period of the treatment, leukopenia and thrombocytopenia were encountered in 20 (80%) and four (16%) patients, respectively. Leukopenia was mild (2000–3000 cells/mm^3) in two patients, moderate (1000–2000 cells/mm^3) in ten patients, and severe (< 1000 cells/mm^3) in eight patients, whereas thrombocytopenia was moderate (50,000–100,000 platelets/mm^3) in three patients and severe (< 50,000 platelets/mm^3) in one. Treatment-related death occurred in the patient with severe thrombocytopenia. Among patients with severe leukopenia, the treatment was stopped in one, and another had pneumococcal meningitis.

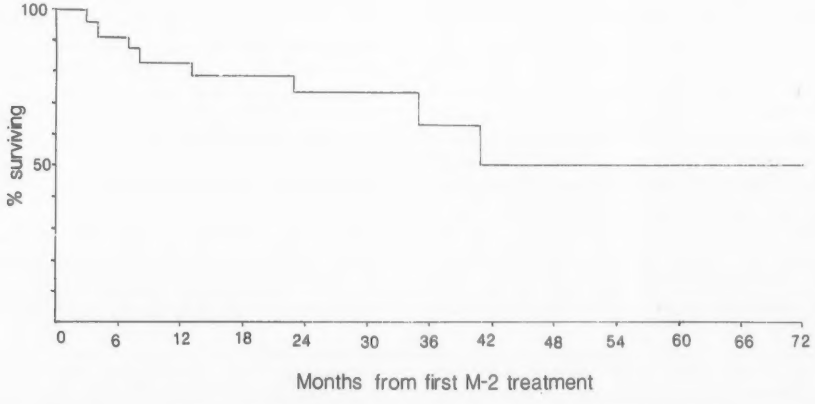


FIGURE 1.—Survival of patients from first M-2. The Nos. of patients at risk at Months 0, 6, 12, 18, 24, 30, 36, and 42 are 25, 23, 21, 14, 9, 6, 3, and 1, respectively.

DISCUSSION

Our results obtained in a group of 25 consecutive patients with previously untreated and symptomatic disease seem to confirm the validity of the M-2 protocol in MM. In particular, the high remission rate, the median time to response, and the length of survival agree with the Memorial Hospital data (1). However, the drop in early mortality in the first 15 months from diagnosis, as reported by the Memorial Hospital group (1), was not supported by our analysis from the start of therapy (5% versus 20%, respectively). Moreover, the grade of toxicity we observed seems higher, although careful reductions of drug dosages have been made, when necessary. In our study, survival was calculated from the beginning of the M-2 protocol treatment, instead of from diagnosis, as done at Memorial Hospital. In the latter way, it could be that patients with asymptomatic MM were not treated for many months, thus improving the early part of a survival curve measured from diagnosis. Moreover, our group of patients have been treated in a large general hospital, not at a referral center such as Memorial Hospital, where subsequent possible selection of patients may have occurred. Nevertheless, the conclusions of the Memorial Hospital group that the M-2 protocol is a more effective treatment than single therapy or melphalan-prednisone therapy on the basis of historical comparisons (1) may be supported even by our results. However, a randomized study comparing melphalan, prednisone, cyclophosphamide, and BCNU (four of the drugs present in the M-2 protocol) with melphalan plus prednisone did not show any significant difference (8). The M-2 protocol includes vincristine, a drug able to add 9 months to the responses obtained with alkylating agent combinations (11) and to induce a further reduction of tumor cells once the labeling index has increased (12).

In conclusion, the Eastern Cooperative Oncology Group randomized study comparing vincristine, BCNU, melphalan, cyclophosphamide, and prednisone with melphalan plus prednisone could clarify the real impact of the M-2 protocol on the treatment of MM.

REFERENCES

1. Case DC, Lee BJ, and Clarkson BD. Improved survival times in multiple myeloma treated with melphalan, prednisone, cyclophosphamide, vincristine and BCNU: M-2 protocol. Am J Med 63:897–903, 1977.
2. Committee of the Chronic Leukemia-Myeloma Task Force, National Cancer Institute. Proposed guidelines for protocol studies. II. Plasma cell myeloma. Cancer Chemother Rep Part 3, vol 4(1):145–158, 1973.
3. Miller S, and Perrotta A. Multiple myeloma: results with the M-2 protocol in a community hospital. Blood (suppl 1) 52:264, 1978.
4. Buonanno G, Tortarolo M, Valente A, et al. Drug-resistant multiple myeloma. A trial with the M-2 cyclical alkylating agent polichemotherapy. Haematologica 63:45–55, 1978.
5. Case DC. Chemotherapy of plasma-cell myeloma. N Engl J Med 302:407, 1980.
6. Lee BJ, Sahakian G, Clarkson BD, et al. Combination chemotherapy of multiple myeloma with Alkeran, Cytoxan, vincristine, prednisone and BCNU. Cancer 33:533–538, 1974.
7. Paccagnella A, Salvagno L, Chiarion Sileni U, et al. Response and survival in multiple myeloma with CTX, MEL, BCNU, VCR, PRED (M-2 protocol). Proc ASCO 1:C-712, 1982.
8. Bergsagel DE, Bailey AJ, Langley GR, et al. The chemotherapy of plasma cell myeloma and the incidence of acute leukemia. N Engl J Med 301:743–748, 1979.
9. Durie BGM, and Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 36:842–845, 1975.
10. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J Cancer 35:1–39, 1977.
11. Alexanian R, Salmon S, Bonnet J, et al. Combination therapy for multiple myeloma. Cancer 36:1258–1268, 1975.
12. Salmon SE. Expansion of the growth fraction in multiple myeloma with alkylating agents. Blood 45:119–129, 1975.

Results of a Randomized Double-Blind Crossover Trial of Scopolamine Versus Placebo Administered by Transdermal Patch for the Control of Cisplatin-Induced Emesis[1]

Dan L. Longo,* Margaret Wesley, Diane Howser, Susan M. Hubbard, Tom Anderson, and Robert C. Young[2]

We tested the efficacy of scopolamine delivered by transdermal patch in preventing emesis induced by cisplatin as a single agent or in combination. The administration of scopolamine by transdermal patch resulted in acceptable side effects but was ineffective in controlling cisplatin-induced emesis. [Cancer Treat Rep 66:1975–1976, 1982]

Cisplatin is one of the most effective chemotherapeutic agents in the armamentarium of the clinical oncologist. Unfortunately, it is one of the most potent emesis-inducing agents as well. Many antiemetic regimens have been unsuccessful in controlling cisplatin-induced emesis. In the current study, we employed scopolamine in an effort to relieve the nausea and vomiting produced by cisplatin.

The belladonna alkaloid scopolamine, a muscarine antagonist, is attractive as a potential antiemetic because of its efficacy in preventing motion sickness (1). The drug is readily absorbed when administered orally or im, however, it produces a variety of side effects such as mouth dryness, blurred vision, tachycardia, anxiety, and behavioral changes through its parasympatholytic action. It is possible that some of these side effects are due to peak serum levels achieved when scopolamine is delivered orally or im. The Alza Corporation, Palo Alto, CA, developed an adhesive patch impregnated with scopolamine capable of gradually delivering the drug transdermally over a period of about 72 hours.[3] The transdermal patch maintains continuous serum levels of scopolamine in the therapeutic range for the prevention of motion sickness.[3] We compared scopolamine administered by transdermal patch to a placebo patch for the capacity to alleviate cisplatin-induced nausea and vomiting and the incidence of scopolamine-related side effects.

After obtaining informed consent, 45 patients (20 receiving cisplatin, cyclophosphamide, vinblastine, bleomycin, and dactinomycin for testicular cancer; 20 receiving cisplatin, 5-FU, cyclophosphamide, and hexamethylmelamine for ovarian cancer; and five receiving cisplatin for breast cancer) were randomized to receive scopolamine or placebo by patch on cycle 1 of treatment and automatically crossed over to the other agent on cycle 2. Patients were stratified according to their underlying malignancy and the chemotherapy they were to receive. Each patient placed a test patch behind each ear 12 hours preceding the iv administration of cisplatin and left the patches in place for 72 hours. Supplemental antiemetics were given on request beginning no sooner than 3 hours after administration of cisplatin. The antiemetic given was thiethylperazine at a dose of 10 mg orally or im every 4–6 hours as needed. Evaluation of drug efficacy and side effects was determined by review of subjective answers to a questionnaire completed by the patient (a copy of the questionnaire is available on request) and by observation of the patient for the presence or absence of vomiting and parasympatholytic physical signs. The questionnaire included assessment of the degree of nausea, the number of vomiting episodes, the number of antiemetic pills or suppositories taken, effects on appetite, the presence of side effects (including drowsiness, dry mouth, blurred vision, rapid heart beat, and dysphoria), and the subjective evaluation of the degree to which the nausea and vomiting interfered with normal living. The questionnaire covered the 72-hour period after treatment with cisplatin chemotherapy. In addition, the patient's vital signs and

[1]Received Feb 1, 1982; revised Aug 20, 1982; accepted Sept 1, 1982.

[2]Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]Shaw J. Data on file with the Alza Corporation, Palo Alto, CA.

*Reprint requests to: Dan L. Longo, MD, Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, MD 20205.

TABLE 1.—Scopolamine compared to placebo in preventing chemotherapy-induced emesis

Diagnosis	No. of evaluable patients (median age)	Treatment program	Median No. of vomiting episodes (range)	
			Scopolamine*	Placebo*
Ovarian cancer	15 (52 yrs)	Cyclophosphamide, 150 mg/m² orally on Days 1–15; hexamethylmelamine, 150 mg/m² orally on Days 1–15; 5-FU, 600 mg/m² iv on Days 1 and 8; and cisplatin, 30 mg/m² iv on Days 1 and 8	13 (1–134)	14 (2–65)
Testicular cancer	11 (23 yrs)	Cyclophosphamide, 1000 mg/m² iv on Day 1; vinblastine, 4 mg/m² iv on Day 1; dactinomycin, 1 mg/m² iv on Day 1; bleomycin, 15 units/m² iv on Day 1; cisplatin, 100 mg/m² iv on Day 7	15 (5–45)	11 (2–45)
Breast cancer	4 (48 yrs)	Cisplatin, 70 mg/m² iv every 21–28 days	8 (7–11)	12 (2–21)

*The order of administration was randomized and the test patches were always placed 12 hrs before the iv administration of cisplatin.

TABLE 2.—Side effects of transdermal patches

Side effects	No. of patients who experienced side effects with—			
	Scopolamine and placebo	Scopolamine only	Placebo only	Neither
Dry mouth	20 (54%)	11 (30%)	2 (5%)	4 (11%)
Blurred vision	5 (14%)	14 (38%)	0 (0%)	18 (49%)
Tachycardia	2 (5%)	7 (19%)	4 (11%)	24 (65%)
Dysphoria	4 (11%)	8 (22%)	4 (11%)	21 (57%)
Palpitations	4 (11%)	3 (8%)	2 (5%)	28 (76%)
Drowsiness	18 (49%)	4 (11%)	6 (16%)	9 (24%)
Behavioral changes	3 (8%)	2 (5%)	0 (0%)	32 (86%)
Fainting and dizziness	1 (3%)	2 (5%)	3 (8%)	31 (84%)

pupil response were measured every 4 hours while awake for the first 36 hours, and the number, times, and volume of emesis were recorded.

Thirty patients (67%) were evaluable to examine the question of antiemetic efficacy and 37 (82%) were evaluable to examine the side effects of the transdermal patch. The major reason patients were not evaluable was failure to complete the questionnaire or the use of qualitative answers such as "a lot" where quantitative data were requested. The statistical analysis was done by the techniques described by Koch (2) for nonparametric methods of looking at the two-period changeover design. There was no evidence for residual effects of one agent carrying over into the test period of the second agent, and the order in which the drug and placebo were given did not affect outcome. Using two-sided tests for significance, scopolamine was found not to be significantly more effective than placebo at preventing chemotherapy-induced emesis for the whole population or any subset of patients (table 1). However, two patients experienced dramatic reduction in the number of vomiting episodes with scopolamine as did two patients receiving placebo. There were no features that identified patients more likely to respond.

The side effects of the transdermal patches are listed in table 2. Most patients reported dry mouth and drowsiness whether they received scopolamine or placebo. Patients were more likely to have dry mouth on scopolamine (two-sided test; $P = 0.02$), but 61% of placebo-treated patients had dry mouth. Patients were more likely to have blurred vision on scopolamine (two-sided test; $P = 0.001$), but 13% of patients had blurred vision on placebo. There were no differences between scopolamine- and placebo-treated groups in the incidence of tachycardia, dysphoria, palpitations, drowsiness, behavioral changes, fainting, or dizziness.

The transdermal patch method of delivering scopolamine does appear to minimize unpleasant side effects. Unfortunately, scopolamine is of limited value in the treatment of cisplatin-induced nausea and vomiting. It is possible that scopolamine may be more useful against emesis induced by other chemotherapeutic agents. Certainly, the efficacy scopolamine has been shown to have in preventing motion sickness (1) and the reduced incidence of side effects it causes when delivered transdermally make it an attractive candidate for further testing.

REFERENCES

1. WOOD CD, and GRAYBIEL A. A theory of motion sickness based on pharmacological reactions. Clin Pharmacol Ther 11:621–629, 1970.
2. KOCH G. The use of nonparametric methods in the statistical analysis of the two-period changeover design. Biometrics 28:577–584, 1972.

Salvage Chemotherapy for Non-Hodgkin's Lymphoma[1]

R. P. Ng, D. Todd,* and R. K. K. Khoo[2,3]

Nineteen patients with advanced non-Hodgkin's lymphoma resistant to previous chemotherapy were treated with combination chemotherapy consisting of lomustine (CCNU), cytarabine, and prednisone. Eight patients (42%) had complete remission and four (21%) had partial remission. The median survival time of the 19 patients was > 14 months. The eight patients who had complete remission were still alive as of May 1, 1982, with a median follow-up time of 632 days (range, 365–1247). We believe that this combination chemotherapy scheme warrants further investigation. [Cancer Treat Rep 66:1977–1979, 1982]

Unsuccessful initial chemotherapy for advanced non-Hodgkin's lymphoma is often associated with a poorer response to subsequent therapy (1). A review of the literature shows that the remission rates and survival times for patients who had had prior chemotherapy were generally lower than those for patients who had not received prior chemotherapy (2–4).

Most chemotherapy regimens consist of cyclophosphamide, vincristine, and prednisone, with the addition of doxorubicin, bleomycin, and/or methotrexate with leucovorin rescue (2–7). Because cytarabine (Ara-C) and lomustine (CCNU) can each produce a response rate of about 20%–30% in advanced non-Hodgkin's lymphoma (8) and because these drugs are not generally used in most of the initial combination chemotherapy schemes, we have investigated the use of the combination of CCNU, Ara-C, and prednisone in patients with non-Hodgkin's lymphoma who had either failed to achieve complete remission (CR) following initial chemotherapy or had had a relapse after initial response. This is a preliminary report of the results.

PATIENTS AND METHODS

Nineteen patients who had stage III/IV non-Hodgkin's lymphoma and failed induction or had a relapse were started on the PAC regimen, which consisted of prednisone (80 mg/m^2/day orally for 5 days starting on Day 1); Ara-C (100 mg/m^2/day sc for 5 days starting on Day 1); and CCNU (40 mg/m^2 orally on Day 1). This regimen was administered every 4 weeks.

Chemotherapy was not restarted for an additional week in the presence of significant thrombocytopenia (< 80,000 cells/mm^3) and/or leukopenia (< 3000 cells/mm^3).

Patient characteristics, including details of disease, treatment, and sites of involvement, are shown in table 1. Except for patient 16, none of the patients had blast cells in the peripheral blood.

All biopsy specimens were classified according to the criteria of Rappaport (9), and performance status was classified according to the World Health Organization classification (10). CR is defined as the absence of all clinical and radiologic evidence of disease and, in those with bone marrow involvement, the absence of evidence of disease on bilateral iliac crest trephine biopsy. Partial remission (PR) is defined as a > 50% reduction in measurable tumor size, and in the cases where there was initial marrow involvement, the absence of clinical evidence of disease but with residual marrow infiltration. Patients who achieved CR were maintained on courses of PAC every 2 months for a maximum of six courses.

RESULTS

The patients' responses to chemotherapy are listed in table 1.

[1]Received Mar 15, 1982; revised July 23, 1982; accepted Aug 5, 1982.

[2]Department of Medicine, University of Hong Kong (*R. P. Ng* and *D. Todd*), and Institute of Radiotherapy and Oncology (*R. K. K. Khoo*), Queen Mary Hospital, Hong Kong.

[3]We thank Dr. F. C. S. Ho, Department of Pathology, Hong Kong University, for her help.

**Reprint requests to:* Prof. D. Todd, Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong.

TABLE 1.—Patient characteristics*

Patient No.	Sex/ age in yrs	Stage	Histology	Performance status	Residual disease site at relapse	Previous treatment	Response to PAC	Survival in days from start of PAC
1	F/68	IV B	DLPD	2	Marrow	CHOP	PR	196
2	F/60	IV A	DLPD	0	Skin	CHOP	CR	456+
3	M/38	IV B	DLPD	1	Marrow	CHOP	CR	826+
4	M/52	IV B	DLPD	0	Skin	CHOP + RT	PR	160+
5	M/52	IV B	DLPD	4	Marrow, pleura	CHOP, CPOB	NR	29
6	F/49	IV B	DLPD	1	Marrow	CHOP	PR	673+
7	F/65	III B	DM	2	Lymph nodes	CHOP	CR	435+
8	M/44	IV B	DM	3	Pleura	CHOP	NR	54
9	M/54	IV B	DM	1	Marrow	HOP	CR	690+
10	F/46	IV B	NLPD	0	Marrow	CVP, CHOP	CR	365+
11	F/51	III B	NLPD	1	Lymph nodes	CHOP, COPP	CR	768+
12	F/51	IV A	DLWD	2	Marrow	CVP, CHOP	PR	329
13	F/61	IV B	Undif	4	Marrow, CNS	CHOP	NR	100
14	M/20	IV A	Undif	3	Marrow, skin	HOP, COPP	NR	115
15	F/44	IV B	DH	1	Marrow	CHOP	CR	573+
16	M/23	IV B	DH	3	Marrow	CHOP	NR	81
17	M/68	IV B	DH	2	Liver, CNS	CHOP	NR	31
18	M/68	IV A	DH	3	Peritoneal seedlings	CHOP	NR	53
19	M/44	III B	DH	3	Lymph nodes	CHOP, CPOB	CR	1247+

*DLPD = diffuse lymphocytic poorly differentiated; DM = diffuse mixed histiocytic/lymphocytic; NLPD = nodular lymphocytic poorly differentiated; DLWD = diffuse lymphocytic well-differentiated; Undif = undifferentiated; and DH = diffuse histiocytic. CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; RT = radiotherapy; CPOB = cyclophosphamide, prednisone, vincristine, and bleomycin; HOP = doxorubicin, vincristine, and prednisone; CVP = cyclophosphamide, vincristine, and prednisone; and COPP = cyclophosphamide, vincristine, procarbazine, and prednisone. NR = no response. + = still alive.

Of the 19 patients, eight (42%) achieved CR, four (21%) had PR, while seven (37%) showed no response. Of the seven who did not show any response to PAC, six were severely ill with advanced disease and were able to receive only one or two courses of chemotherapy, and all seven have died, with a median duration of survival of 54 days. The eight patients who had CR are still alive as of May 1, 1982, with a median follow-up time of 632 days (range, 365–1247). Only two patients (Nos. 9 and 15) had evidence of relapse. Using life-table analysis the 50% probability of survival for the 19 patients is > 14 months.

The major side effects were nausea, vomiting, and marrow suppression. The nausea and vomiting was made tolerable by administration of concomitant metoclopramide. A total of 96 courses of PAC have been administered to these 19 patients; 11 episodes of leukopenia < 2000 cells/mm^3 and six episodes of thrombocytopenia < 100,000 cells/mm^3 were recorded. There were five episodes of both leukopenia and thrombocytopenia. The blood count nadirs usually occurred in weeks 3–4, mostly after the third course of chemotherapy. There was no death due to drug toxicity.

DISCUSSION

Results of chemotherapy in patients with non-Hodgkin's lymphoma who had had previous chemotherapy have generally been poor (1–4), and the response rates and survival times in our study compare favorably with these previous reports. Because CCNU is given orally and Ara-C is given sc, the PAC regimen can be readily administered in the outpatient clinic. This implies that the quality of life of these patients on the whole is better than that of patients whose chemotherapy must be administered in the hospital and in a protective environment. The drugs in the PAC regimen are also relatively inexpensive.

The dose of CCNU used in this study was smaller than the conventional dose reported in the literature, based on our experience with cytotoxic drugs in Chinese patients (unpublished observation) and based on the administration of CCNU once every 3–4 weeks rather than the usual 6 weeks.

The effectiveness, ease of administration, and relatively low cost of PAC make this an attractive regimen, and we believe that it warrants further trial as a salvage method.

REFERENCES

1. DURANT JR, LOEB V, DORFMAN R, ET AL. 1,3-Bis(2-chloroethyl)-1-*N*-nitrosourea (BCNU), cyclophosphamide, vincristine and prednisone (BCOP). Cancer 36:1936–1944, 1975.
2. SCHEIN PS, DEVITA VT, JR, HUBBARD S, ET AL. Bleomycin, adriamycin, cyclophosphamide, vincristine, and prednisone (BACOP) combination chemotherapy in the treatment of advanced diffuse histiocytic lymphoma. Ann Intern Med 85:417–422, 1976.
3. SWEET DL, GOLOMB HM, UTLMANN JE, ET AL. Cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine (COMLA) combination sequential chemotherapy for advanced diffuse histiocytic lymphoma. Ann Intern Med 92:785–790, 1980.
4. ELIAS L, PORTLOCK CS, and ROSENBERG SA. Combination chemotherapy of diffuse histiocytic lymphoma with cyclophosphamide, adriamycin, vincristine and prednisone (CHOP). Cancer 42:1705-1710, 1978.
5. SCHEIN PS, CHABNER BA, CANELLOS GP, ET AL. Potential for prolonged disease free survival following combination chemotherapy of non-Hodgkin's lymphoma. Blood 43:181–189, 1974.
6. BAGLEY C, DEVITA VT, JR, BERARD CW, ET AL. Intensive cyclical chemotherapy with cyclophosphamide, vincristine and prednisone. Ann Intern Med 76:227–234, 1972.
7. SKARIN AT, FREI E, III, MOLONEY WC, ET AL. New agents and combination chemotherapy of non-Hodgkin's lymphomata. Br J Cancer 31:497–504, 1975.
8. BONADONNA G, LATTUADA A, and BANFI A. Recent trends in the treatment of non-Hodgkin's lymphoma. Eur J Cancer 12:661–673, 1976.
9. RAPPAPORT H. Tumors of the hematopoietic system. *In* Atlas of Tumor Pathology, sec III, fascicle 8. Washington, DC, US Armed Forces Institute of Pathology, 1966, pp 91–206.
10. WHO Handbook for Reporting Results of Cancer Treatment. Geneva, WHO, 1979, p 7.

Ip Immunotherapy and Chemotherapy in Advanced Epithelial Ovarian Cancer[1,2]

Enrique Hernandez,* Neil B. Rosenshein, Jose Pino y Torres, Jose Villar, Francis C. Grumbine, David S. Ettinger, Michael B. Dillon, Jerry L. Klein, and Stanley E. Order[3]

Nine patients with advanced epithelial ovarian cancer, classified as either stage III with macrometastatic residual disease or stage IV, received combination chemotherapy in conjunction with ip rabbit-derived human ovarian antitumor serum (HOATS). The HOATS regimen consisted of the ip instillation of 100 ml of ovarian antitumor serum on Days 1 and 3 of the first chemotherapy cycle. Side effects attributable to HOATS were low-grade fever in three patients and diffuse skin rash in two. The 1-year cumulative survival was 87%, with a clinical response rate of 80%. No significant toxic reactions to HOATS have been observed. Continuation of the present study seems justified. [Cancer Treat Rep 66:1981–1982, 1982]

Advanced ovarian carcinoma (stages III and IV) is almost uniformly fatal, with a 5-year survival rate of < 10% (1). Therapeutic management typically consists of tumor-reductive surgery, followed by either chemotherapy, radiotherapy, or chemotherapy plus nonspecific immunotherapy (1–8). Systemic chemotherapy is currently the preferred treatment modality for patients with advanced disease, as the results with radiotherapy in patients with large residual tumors have been discouraging (9,10).

A new approach to the management of this disease is the administration of ip immunotherapy in combination with multiagent chemotherapy. The use of these two modalities seeks to take advantage of the improved response observed in an ovarian cancer model when chemotherapy was combined with tumor-specific immunotherapy (11). Preliminary data on the use of this unique technique are presented.

METHODS

The study population consisted of patients with stage III or IV epithelial ovarian adenocarcinoma staged according to the International Federation of Obstetrics and Gynecology classification. Stage III was subdivided into micrometastatic (residual tumor nodules measured < 5 mm and covered < 50% of the peritoneal cavity) and macrometastatic (residual tumor nodules measured ≥ 5 mm or covered ≥ 50% of the peritoneal cavity). This report deals only with patients having stage III macrometastatic or stage IV disease, with histology of serous, mucinous, or undifferentiated adenocarcinomas and with no prior history of receiving chemotherapy or radiation. All patients were fully active at the initiation of therapy.

The chemotherapy regimen, given on a 28-day cycle, consisted of 150 mg/m^2 of hexamethylmelamine given orally on Days 1–14 and 500 mg/m^2 of cyclophosphamide given iv on Day 1, with 40 mg/m^2 of doxorubicin given iv on Day 1 alternating with 50 mg/m^2 of cisplatin given iv on Day 1 every other cycle. Prior to the initiation of chemotherapy, all patients had adequate hematologic, renal, and liver functions.

Human ovarian antitumor serum (HOATS) is prepared from patients with ovarian adenocarcinoma of mucinous, serous, or undifferentiated type. The technique of HOATS preparation has been previously described (12). The treatment regimen consisted of initial skin and eye testing followed by the ip instillation of 100 ml of HOATS on Days 1 and 3 of the first chemotherapy cycle. This was done by placing an intra-abdominal catheter, demonstrating free peritoneal flow with iodinated contrast material, and then infusing the HOATS.

[1]Received Mar 18, 1982; revised July 16, 1982; accepted Aug 5, 1982.

[2]Supported in part by Public Health Service grants CA-25905 and CA-17970 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a Clinical Fellow Award to Dr. Enrique Hernandez from the American Cancer Society.

[3]Divisions of Gynecologic Oncology (*E. Hernandez, N. B. Rosenshein, J. Villar, F. C. Grumbine,* and *M. B. Dillon*), Radiation Oncology (*J. Pino y Torres, J. L. Klein,* and *S. E. Order*), and Medical Oncology (*D. S. Ettinger*), The Johns Hopkins Medical Institutions, Baltimore, MD.

**Reprint requests to:* Enrique Hernandez, MD, Division of Gynecologic Oncology, The Johns Hopkins Hospital, 600 N Wolfe St, Baltimore, MD 21205.

Informed consent was obtained and the patients were then stratified by performance status, tumor grade, and histology, and randomized to receive or not receive HOATS in conjunction with the chemotherapy.

RESULTS

From January 1980 to July 1981, nine patients who met the above criteria were treated with HOATS and chemotherapy. Initially, four patients received HOATS as part of the pilot program. Five patients received HOATS as part of the ongoing prospective randomized study. Clinically evaluable disease was present in five patients. A clinical response was documented in four patients (complete response in 40%, partial response in 40%). The 1-year cumulative survival for these patients was 87%. The observed side effects attributable to HOATS were low-grade fever in three patients and diffuse skin rash in two. No significant increase in bone marrow toxicity was noted in this group of patients.

DISCUSSION

In the past three decades, there has been no significant improvement in the survival of patients with ovarian cancer. If we are to improve the survival of these patients, unique forms of therapy need to be explored. Our present HOATS protocol is such an effort.

It has been demonstrated in an ovarian cancer model that host sensitization by antiserum was therapeutic if host macrophages were available (13–15). In addition, an improved response was noted in this model when chemotherapy was combined with antiserum (11). The combination of HOATS and chemotherapy seeks to take advantage of these observations.

HOATS consists of polyclonal antibodies produced against multiple antigenic moieties found on the epithelial ovarian tumors. These antigenic moieties have also been identified in normal tissues (16). The administration of HOATS ip enables the antibodies to preferentially attach to the intraperitoneal cancer rather than to the similar antigenic moieties found in the circulation. As HOATS is a foreign protein, the antibody-antigen complex formed stimulates the host's immune system, activating intraperitoneal macrophages.

We have encountered no significant toxic reactions to HOATS, and patient survival has not been compromised. It seems justified to continue with the stratified randomized study.

REFERENCES

1. Willson JKV, Ozols RF, Lewis BJ, et al. Current status of therapeutic modalities for treatment of gynecologic malignancies with emphasis on chemotherapy. Am J Obstet Gynecol 141:81–98, 1981.
2. Edmonson JH, Fleming TR, Decker DG, et al. Different chemotherapeutic sensitivities and host factors affecting prognosis in advanced ovarian carcinoma versus minimal residual disease. Cancer Treat Rep 63:241–247, 1979.
3. Griffiths CT, Grogan RH, and Hall TC. Advanced ovarian cancer: primary treatment with operation, radiotherapy and chemotherapy. Cancer 29:1–7, 1972.
4. Griffiths CT. Surgical resection of tumor bulk in the primary treatment of ovarian carcinoma: symposium on ovarian cancer. Natl Cancer Inst Monogr 42:101–104, 1975.
5. Griffiths CT, and Fuller AF. Intensive surgical and chemotherapeutic management of advanced ovarian cancer. Surg Clin North Am 58:131–142, 1978.
6. Griffiths CT, Parker LM, and Fuller AF, Jr. Role of cytoreductive surgical treatment in the management of advanced ovarian cancer. Cancer Treat Rep 63:235–240, 1979.
7. Wijnen JA, and Rosenshein NB. Surgery in ovarian cancer. Arch Surg 115:863–868, 1980.
8. Alberts DS, Moon TE, Stephens RA, et al. Randomized study of chemoimmunotherapy for advanced ovarian carcinoma: a preliminary report of a Southwest Oncology Group study. Cancer Treat Rep 63:325–331, 1979.
9. Delclos L, and Quinlan EJ. Malignant tumors of the ovary managed with postoperative mega voltage irradiation. Radiology 93:659–663, 1969
10. Dembo AJ, Bush RS, Beale RA, et al. Ovarian carcinoma: improved survival following abdominopelvic irradiation in patients with a completed pelvic operation. Am J Obstet Gynecol 134:793–800, 1979.
11. Wright T, Sinanan M, Harrington D, et al. Immunoglobulin: applications to scanning and treatment. Appl Radiol 8(2):120–124, 1979.
12. Order SE, Rosenshein NB, Klein JE, et al. The integration of new therapies and radiation in the management of ovarian cancer. Cancer 48:590–596, 1981.
13. Order SE, Kirkman R, and Knapp R. Serologic immunotherapy: results and probable mechanism of action. Cancer 34:175–183, 1974.
14. Order SE, Donahue V, and Knapp R. Immunotherapy of ovarian carcinoma—an experimental model. Cancer 32:573–579, 1973.
15. Vanhaelen CP, Fisher RI, Apella E, et al. Lack of histocompatibility antigens on a murine ovarian teratocarcinoma. Cancer Res 41:3186–3191, 1981.
16. Order SE, Thurston J, and Knapp R. Ovarian tumor antigens: a new potential for therapy. Natl Cancer Inst Monogr 42:33–43, 1975.

Autologous Bone Marrow Transplantation in Acute Myeloid Leukemia in Relapse or in Complete Remission[1]

P. Herve,[2,*] A. Rozenbaum,[3] E. Plouvier,[3] M. Flesch,[3] J. Y. Cahn,[3] A. Farradji,[4] J. P. Bergerat,[4] J. C. Adjizian,[5] and B. Pignon[5,6]

We report ten cases of acute myeloid leukemia treated by intensive therapy followed by autologous bone marrow transplantation. Seven patients were in first relapse, and three were in complete remission. The conditioning regimen consisted of either chemotherapy alone (6-thioguanine, cytarabine, lomustine, and cyclophosphamide [TACC; eight patients]) or cyclophosphamide and total-body irradiation (two patients). All the patients in first relapse achieved complete remission (CR). The median remission duration was 9.9 months (range, 5–14), and the median survival was 14.4 months (range, 9–23.8). Of the three patients autografted during CR, one relapsed at Month 5 and two others remain in CR and are well at 18+ and 18.3+ months. High-dose chemotherapy followed by autologous bone marrow transplantation seems to be a valuable treatment for acute myeloid leukemia, if it is used immediately after the CR to consolidate the remission. [Cancer Treat Rep 66:1983–1985, 1982]

Autologous bone marrow transplantation (ABMT) following high-dose chemotherapy with or without total-body irradiation (TBI) represents a new approach to the treatment of patients with acute myeloid leukemia (AML) without HLA-identical donors, either when conventional therapy has failed or to consolidate and hopefully to prolong a complete remission (CR). In this paper, we report the antitumor effect of high-dose chemotherapy in ten patients with AML. They were entered into our regional therapeutic protocol between April 1979 and June 1981.

PATIENTS AND METHODS

Patients.—The group consisted of ten patients (eight adults and two children) with AML. Subtypes of AML, according to the FAB classification (1), were as follows: M_2 (seven patients), M_1 (two), and M_6 (one). The median age was 28.2 years (range, 2–45). After diagnosis, all patients received extensive induction and consolidation therapy, and four of seven patients grafted in relapse had received maintenance therapy (table 1). Nine patients had marrow collection and storage during the first CR, and one during the second CR. Seven patients had high-dose chemotherapy and ABMT during the first relapse, and three others, during the first remission.

Bone marrow processing and cryopreservation.—These methods have already been described (1–3). Briefly, the bone marrow was enriched with a blood separator (Haemonetics model 30). DMSO in plasma was added to a final 10% concentration. The marrow cells were frozen at a slow rate in a freezer with an electronic programmer and stored in liquid nitrogen. After thawing, a stepwise dilution was employed followed by a centrifugation allowing the removal of supernatant with hemoglobin and DMSO. The marrow cells were infused immediately.

Conditioning of patient for marrow transplantation.—The conditioning regimen consisted either of a high-dose chemotherapy combination containing 6-thioguanine, cytarabine, lomustine, and cyclophosphamide (TACC; eight patients) (4) or of cyclophosphamide plus 10-Gy TBI (two patients). We modified the TACC regimen by increasing the dose of 6-thioguanine from 200 to 400 mg/m^2 daily over 4 days, of cytarabine from 200 to 400 mg/m^2 daily over 4 days, and of lomustine

[1]Received Mar 22, 1982; revised July 13, 1982; accepted Aug 5, 1982.
[2]Centre Régional de Transfusion Sanguine, Besançon, France.
[3]Cliniques d'Hématologie, Hôpital Saint-Jacques, Besançon, France.
[4]Clinique d'Hématologie, Hôpital de Hautepierre, Strasbourg, France.
[5]Laboratoire d'Hématologie, Service de Médecine, Hôpital Robert Debré, Reims, France.
[6]We thank Miss E. Tamayo and Mr. A. Jeanroy for excellent technical assistance, and Miss E. Genevois for excellent secretarial help.
*Reprint requests to: P. Herve, MD, Centre Régional de Transfusion Sanguine, 15, rue Mégevand, BP 1181, F 25003 Besançon Cedex, France.

TABLE 1.—Clinical characteristics of patients, response of leukemia, and follow-up after ABMT

Patient No.	FAB classification	Age (yrs) and sex	Time from onset of leukemia (mos)	Past drug therapy*	Maintenance treatment	Clinical status at time of ABMT	Conditioning regimen†	Response	Outcome	Survival (mos)
1	M_2	45 M	16	CA, DNR, MTX, 6MP	Yes	1st relapse	TACC	CR	Relapse (13th mo)	23.8
2	M_2	35 M	3	CA, DNR, VCR, 6TG	No	1st relapse	Cy + TBI	CR	Relapse (5th mo)	7.3
3	M_2	22 F	32	CA, DNR, 6TG, 6MP	Yes	1st relapse	TACC	CR	Relapse (12th mo)	14
4	M_1	25 M	9	CA, DNR, 6TG, 6MP	Yes	1st relapse	TACC	CR	Relapse (13th mo)	15.8
5	M_2	17 F	6	CA, DNR, 6TG	No	1st relapse	Cy + TBI	CR	Relapse (5th mo)	9
6	M_2	43 F	8	CA, DNR, 6TG	No	1st relapse	TACC	CR	Relapse (6.5th mo)	16
7	M_2	39 F	27	CA, DNR, 6TG	Yes	1st relapse	TACC	CR	Relapse (14th mo)	18+
8	M_6	44 M	6	CA, DNR, 6TG	No	1st CR	TACC	—	CR	18.3+
9	M_1	10 F	4	CA, DNR, 6TG	No	1st CR	TACC	—	CR	18+
10	M_2	2 M	6	VCR, CA, DNR, 6TG, Pred	No	2nd CR	TACC	—	Relapse (5th mo)	12+

*CA = cytarabine, DNR = daunorubicin, MTX = methotrexate, 6MP = 6-mercaptopurine, 6TG = 6-thioguanine, VCR = vincristine, and Pred = prednisone.
†Cy = cyclophosphamide.

from 200 to 400 mg/m^2 over 1 day. Cyclophosphamide was given daily at the same dose of 45 mg/kg over 4 days (5).

RESULTS

Harvesting Data

In the ten patients, the median net volume of aspirate was 898 ml (range, 375–1250). The mean yield of nucleated cells was 1.16×10^{10} (range, 0.6–2.4). After concentration with a blood cell separator, the pool of buffy coat was only 20% of the original volume and contained 57.6% ± 15.54% (mean ± SD) of the total cells; 79% ± 9.2% (mean ± SD) of the CFU-GM were recovered in the final concentrate. The average total CFU-GM cryopreserved was 2.75×10^6 (range, 0.2–8.8).

Toxicity of the Conditioning Regimen

Two severe cardiac complications occurred during initial treatment: one patient who died within 6 days of ABMT is not included in this study; the other patient (No. 7) developed congestive heart failure within 10 days of ABMT, which responded to treatment. Gastrointestinal symptoms were observed in almost every patient (nausea, vomiting, and diarrhea). Stomatitis was seen in two patients. No side effects were observed after transfusion of thawed marrow cells, except for three patients who developed fever 1 hour after reinfusion.

Kinetics of Hematopoiesis Recovery

Six patients achieved granulocyte counts > 0.5×10^9/L between Days 14 and 20. In three patients (Nos. 2, 5, and 6), hematopoietic recovery was delayed. Granulocyte counts > 0.5×10^9/L were reached between Days 35 and 45, and platelet counts > 50×10^9/L were reached between Days 40 and 80. Two of these patients had received a low number of nucleated cells (0.98 and 1.2×10^8/kg). For patients 8 and 9, grafted in CR, we observed a very delayed platelet recovery, though the number of nucleated cells infused was considered adequate.

Antileukemia Effects (Table 1)

All seven patients treated in first relapse with TACC or cyclophosphamide + TBI achieved a second CR. The median remission duration was 9.9 months (range, 5–14). The addition of TBI to the chemotherapy did not improve the duration of second CR for patients 2 and 5. The median survival was 14.4 months (range, 9–23.8). Only one patient (No. 7) is still alive (18+ months). The second CR was > 10 months in four of the seven patients. For five of the seven patients, survival was ≥ 1 year. The quality of life after ABMT was good, and most patients treated with chemotherapy alone and

achieving hematopoietic recovery stayed in the hospital for a short time (30 days on average).

Of the three patients treated during CR, two are in CR and well (18+ and 18.3+ months), without any maintenance treatment. Patient 10 relapsed at the fifth month but achieved a third CR after high-dose melphalan (200 mg/m^2) followed by rescue with thawed bone marrow collected 2 months before. He has remained in his third CR for 7 months.

DISCUSSION

Case reports show that high-dose cytoreductive regimens followed by ABMT can induce CR in relapsed acute leukemia. The TACC regimen appeared effective when employed early in relapse. The patients were not selected; however, in all cases but one (patient 10), the bone marrow was collected early in the course of the first remission.

For AML, the incidence of CR has improved during recent years, but a very small proportion of the patients who achieve CR can expect to be cured. Duration of the first CR in AML still remains short.

Intensive cytoreductive treatment followed by ABMT can be used in two different situations in AML: either in relapse (6) or immediately after CR is reached, to consolidate and possibly increase duration of remission. After ABMT, maintenance chemotherapy can be avoided and the quality of life is better.

For the AML patients grafted during CR, a comparative study of the antileukemic effect of two different conditioning regimens, such as chemotherapy alone versus TBI plus cyclophosphamide, must be evaluated. However, the toxicity of the conditioning regimen must be taken into account before a patient in CR is entered into this protocol. We have had a patient treated during CR who died before marrow grafting because of cardiac failure. A complete evaluation of risks should be made and the patient should be informed of them before agreement to the procedure. More patients treated earlier in CR are needed to compare this treatment modality, in terms of survival, with conventional chemotherapy.

One fundamental problem remains: the bone marrow collected from leukemic patients during CR for autotransplantation contains residual malignant cells. Various approaches are proposed to remove leukemic cells. The bone marrow may be treated with cytotoxic drugs in vitro just before the cryopreservation step. Sharkis et al (7) have proposed the use of 4-hydroperoxycyclophosphamide, and we have started an ABMT protocol using this drug in vitro.

In conclusion, marrow viability after thawing and hematopoietic reconstitution even after TBI have been established, though in some cases the full reconstitution is delayed. However, several unresolved problems remain: (*a*) the effect of freezing on the megakaryocyte stem cell (platelet recovery is often delayed compared to granulocyte recovery); (*b*) the control of the nonmedullary toxic effects of intensive chemotherapy; (*c*) the antileukemic effect of chemotherapy alone versus chemotherapy plus TBI; and (*d*) the means to remove the residual leukemic cells from harvested bone marrow.

REFERENCES

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of acute leukemia. Br J Haematol 33:451–458, 1976.
2. Schaefer NW. Autologous bone marrow transplantation in acute leukemia. A review. *In* Bone Marrow Transplantation in Europe (Touraine JL, ed). Amsterdam and Oxford, Excerpta Medica, 1979, pp 71–79.
3. Herve P, Tamayo E, Coffe C, et al. Cryopreservation of human bone marrow with a view to autologous bone marrow transplantation. *In* Bone Marrow Transplantation in Europe (Touraine JL, ed). Amsterdam and Oxford, Excerpta Medica, 1978, pp 80–87.
4. Gorin NC, David R, Stachowiak J, et al. High dose chemotherapy and autologous bone marrow transplantation in acute leukemias, malignant lymphomas and solid tumors. A study of 23 patients. Eur J Cancer 17:557–568, 1981.
5. Herve P, Rozenbaum A, Plouvier E, et al. Greffe de cellules-souches hématopoïétiques autologues. Premiers résultats d'un protocole régional. Nouv Presse Med 10:2001–2006, 1981.
6. Dicke KA, Spitzer G, Peters L, et al. Autologous bone marrow transplantation in relapsed adult acute leukemia. Lancet 3:514–517, 1979.
7. Sharkis SJ, Santos GW, and Colvin M. Elimination of acute myelogenous leukemic cells from marrow and tumor suspensions in the rat with 4-hydroperoxycyclophosphamide. Blood 55:521–523, 1980.

Multiple Squamous Cell Carcinoma Treated With Intra-arterial Cisplatin in a Patient With Rheumatoid Arthritis[1]

Jaffer A. Ajani* and Michael A. Burgess[2]

A 64-year-old woman, who had had rheumatoid arthritis controlled for many years by prednisone, developed multiple biopsy-proven squamous cell carcinoma of the skin extending from the left elbow to the hand. She was treated with intra-arterial cisplatin (90 mg/m^2) every 4 weeks. After four treatments, she achieved a complete clinical remission that was maintained (8+ months) until she died of an unrelated cause. We believe that arterial infusion of cisplatin as treatment for advanced regional squamous cell carcinoma of the skin should be pursued further as part of a multimodal approach and as definitive therapy. [Cancer Treat Rep 66:1987–1989, 1982]

Squamous cell carcinoma (SCC) is a common tumor, most frequently found on surfaces exposed to ultraviolet radiation. Recent evidence suggests that patients with immunologic defects have a higher than expected incidence of SCC of the skin arising de novo (1–7). Such tumors are not infrequently multiple and may have a more malignant behavior than those arising from preexisting skin lesions (1,8). We describe here a patient with rheumatoid arthritis controlled over many years with steroids, who developed multiple SCC and was successfully treated with intra-arterial infusions of cisplatin.

CASE REPORT

A 64-year-old woman who had had severe rheumatoid arthritis for 39 years was referred to The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute at Houston for treatment of multiple SCC of the skin of the left upper extremity in June 1980. SCC had previously been excised from her left and right ankle regions in 1975 and from her left forearm in May 1980. Within 1 month of the latter surgery, multiple new tumors appeared over the left upper extremity.

The patient's rheumatoid arthritis had been well-controlled for many years by administering prednisone (20 mg/day). Examination revealed multiple joint deformities consistent with the diagnosis of rheumatoid arthritis. Extending from the elbow to the hand, there were approximately ten hard, nontender cutaneous nodules, each measuring < 1 cm in diameter (fig 1A); in June 1980, biopsy was performed on four of the nodules, confirming the diagnosis of SCC. There was no lymphadenopathy in the axillary, cervical, or inguinal regions. The chest X-ray and liver chemistry evaluations did not reveal any evidence to suggest metastatic tumor.

In July 1980, intra-arterial cisplatin (90 mg/m^2) was infused through a catheter in the left brachial artery. A French arterial catheter (size 6.5) was advanced from the femoral artery to the brachial artery for infusion. Half-strength normal saline solution was used for prehydration and posthydration. Cisplatin was mixed in 500 ml of one-half normal saline and was infused over 2 hours. Before cisplatin infusion, 50 ml of 20% mannitol solution was given iv, and during cisplatin infusion, 100 ml of mannitol solution was given iv. Two weeks after the first treatment, the patient was admitted to the hospital with a diagnosis of acute myocardial infarction, from which she recovered uneventfully. Six weeks later the intra-arterial chemotherapy was resumed, every 4 weeks. All tumors regressed. After four treatments the patient was in complete remission (fig 1B). One further treatment was given in December 1980 to consolidate the excellent response. The patient did not develop any

[1]Received June 9, 1982; revised Aug 12, 1982; accepted Aug 19, 1982.
[2]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

*Reprint requests to: Jaffer A. Ajani, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

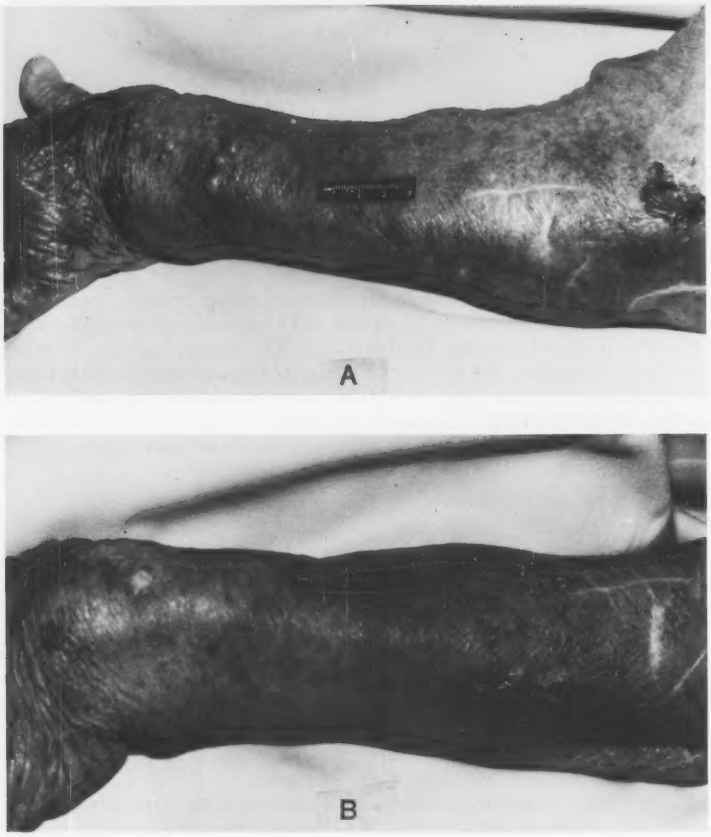


FIGURE 1.—64-yr-old woman treated for rheumatoid arthritis with prednisone developed SCC extending from left elbow to hand (A). After 4 intra-arterial treatments with cisplatin she had complete remission (B).

local or systemic toxic effects as a result of intra-arterial cisplatin.

On July 31, 1981, the patient was admitted to a local hospital, acutely ill and reporting low back pain. Clinically, there was evidence of gas gangrene involving the upper thigh. She developed cardiopulmonary arrest within hours of hospitalization and could not be resuscitated. Autopsy revealed an unsuspected carcinoma of the transverse colon but no evidence of residual SCC of the left upper extremity or anywhere else in the body.

DISCUSSION

Evidence suggests that, in this patient, the development of multiple SCC was related to long-term immunosuppressive therapy rather than to the underlying rheumatic disorder. For patients with extensive SCC, in whom the traditional therapeutic approaches of surgical excision and radiation therapy are not applicable, arterial infusions of chemotherapeutic agents including bleomycin, methotrexate, dactinomycin, vincristine, vinblastine, 5-FU, and hydroxyurea have been used with varying degrees of success (9).

The choice of cisplatin for this patient resulted from our experience with infusing this drug by the arterial route (10,11) and from its established activity against SCC when given systemically (12,13). The complete clinical remission in this patient for 8 months suggests that infusion of cisplatin as treatment for advanced re-

gional SCC should be pursued further, both as a definitive therapy and as part of a multimodal approach. Successful preoperative regional chemotherapy may permit less radical surgical resection (amputation) in some patients.

REFERENCES

1. HEENAN PJ, and DAWKINS RL. Cryptococcosis and multiple squamous cell tumors associated with a T-cell defect. Cancer 47:291–295, 1981.
2. PINKUS H. Cancer of the Skin: Biology-Diagnosis-Management. *In* Actinic Keratosis-Actinic Skin (Andrade R, Gumport SL, Popkin GL, et al, eds). Philadelphia, W. B. Saunders, 1976, p 1661.
3. WALDER BK, ROBERTSON MR, and JEREMY D. Skin cancer and immunosuppression. Lancet 2:1282–1283, 1971.
4. GREENE MH, HOOVER RN, and FRAUMENI JF, JR. Subsequent cancer in patients with chronic lymphocytic leukemia—a possible immunologic mechanism. JNCI 61:337–340, 1978.
5. PENN I. Malignancies associated with immunosuppressive or cytotoxic therapy. Surgery 83:492–502, 1978.
6. LEWIS RB, CASTOR CW, KNISLEY RE, ET AL. Frequency of neoplasia in systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 19:1256–1260, 1976.
7. CANOSO JJ, and COHEN AS. Malignancy in a series of 70 patients with systemic lupus erythematosus. Arthritis Rheum 17:383–390, 1974.
8. TURK LL, and WINDER PR. Carcinomas of the skin and their treatment. Semin Oncol 7:376–384, 1980.
9. STEPHENS FO, and HARKER GJS. The use of intra-arterial chemotherapy for treatment of malignant skin neoplasms. Aust J Dermatol 20:99–107, 1979.
10. PRITCHARD JD, MAVLIGIT GM, BENJAMIN RS, ET AL. Regression of regionally confined melanoma with intra-arterial *cis*-dichlorodiammineplatinum(II). Cancer Treat Rep 63:555–558, 1979.
11. PRITCHARD JD, WALLACE S, MCBRIDE CM, ET AL. Regression of regionally advanced malignant melanoma after arterial infusion with cis-platinum and actinomycin D. Clin Oncol. In press.
12. D'AOUST JC, and ISSELL BF. Cisplatin (*cis*-diamminedichloroplatinum II). Cancer Treat Rev 6:17–39, 1979.
13. KEESLER W. Randomized comparison of *cis*-diamminedichloroplatinum versus *cis*-diamminedichloroplatinum, methotrexate, and bleomycin in recurrent squamous cell carcinoma of the head and neck. Cancer Chemother Pharmacol 3:57–59, 1979.

High-Dose AMSA and Autologous Bone Marrow Transplantation in Relapsed Acute Leukemia[1]

Axel R. Zander,* Lijda Vellekoop, Karel A. Dicke, Michael J. Keating, Kenneth B. McCredie, Sewa S. Legha, Gary Spitzer, Dharmvir Verma, and Gerald P. Bodey[2]

AMSA is a recently developed antitumor agent that has undergone clinical studies in solid tumors and leukemias (1–5). Since its dose-limiting toxic effect is myelosuppression, we undertook a study giving AMSA in high doses (800 mg/m^2) divided in four daily doses to patients with relapsed leukemia, followed by infusion of autologous bone marrow that had been stored during remission.

Eleven patients (median age, 27 years; range, 19–54) received a total of 13 high-dose AMSA treatments with bone marrow rescue. All patients except two had failed reinduction attempted before entering this program (table 1). The performance status ranged from 1 to 3 on the Zubrod scale. A median of 1×10^8 (range, 0.4–1.4×10^8) nucleated bone marrow cells/kg of body weight were infused. The viability of the bone marrow was checked with the in vitro culture of granulocyte macrophage precursors (CFC-GM). A median of 44,720 CFC-GM were infused (range, 3400–152,000). Five patients died during the treatment course with AMSA and bone marrow rescue. Three of the five patients had systemic *Candida* infections, which did not respond to amphotericin B, and died within 3 weeks after transplantation. Two patients died after a prolonged period of bone marrow aplasia (37 and 60 days) without evidence of bone marrow engraftment. In three patients leukemia occurred after an initial period of aplasia. Two of these patients received further investigational treatment. Three patients had responses: one patient (acute myelocytic leukemia) had hematologic improvement of 4 months' duration, one patient (acute lymphocytic leukemia) had a partial remission after the first course and progressing disease following recovery from the second course, and one patient (acute myelocytic leukemia) had a complete remission of 4 months' duration.

Major toxic effects consisted of fever in all 13 courses (11 patients). Systemic fungus infections were documented in three courses, and bacterial infections were documented in four. Nausea and vomiting occurred during all 13 courses, and severe stomatitis occurred in six. Transient liver function abnormalities were associated with systemic infections in six courses. Renal function abnormalities occurred in two patients, subsequent to treatment for systemic fungus infection. No cardiac side effects were noted.

The overall response rate was disappointing, with one complete remission among 11 treated patients. However, only two patients received AMSA as the initial treatment for the first relapse. The low response rate can be explained by extensive previous therapy and corresponds with other observations made with AMSA treatment in refractory leukemia (3). It appears that the infusion of cryopreserved autologous bone marrow was not able to modify the infectious complications of myelosuppression expected in this high-risk patient population. High-dose chemotherapy with autologous bone marrow rescue should be undertaken early in the course of treatment in patients with malignancies sensitive to chemotherapy, when an optimal response with minimal side effects can be expected.

[1]Received Aug 14, 1981; revised July 12, 1982; accepted Aug 30, 1982.

[2]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Axel R. Zander, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Previous treatments and response to high-dose AMSA and autologous bone marrow rescue

Patient No./age (yrs)	Diagnosis*	Previous treatment†	Duration of treatment (mos)	AMSA treatment (mg)	Response	Outcome
1/25	AML	AdOAP → CR; AIM → F, POMP → F; CBV + ABMT → PR	34	800	Aplasia	Died on Day 37 of liver failure
2/28	AML	AdOAP → CR; Pip TBI + ABMT → CR	24	800	Failure	Subsequent chemotherapy
3/28	AMML	AdOAP → CR; ROAP → CR; CBV + ABMT → CR	49	800	Aplasia	Died on Day 19 of fungemia
4/25	ALL	AdOAP → CR; CROP → CR; POMP → F	35	800	Failure	Died on Day 15 of fungemia
5/30	APL	AdOAP → CR; CROP → CR; ara-C + 6-thioguanine → F; AdOAP → CR	24	800	Aplasia	Died on Day 10 of fungemia
6/25	AML	AdOAP → CR	23	800	Aplasia	Died on Day 60 of liver failure
7/40	ALL	AdOAP → CR; OAP	61	800	Partial response	Subsequent chemotherapy
8/22	AML	AdOAP → CR; OAP → CR	18	800 × 2	Failure	Subsequent chemotherapy
9/27	ALL	CHOP-Bleo → CR; AIM → CR; CBV + ABMT → F	32	800 × 2	Failure	Refused further chemotherapy
10/19	AML	AdOAP → CR	18	800	Complete response	Subsequent chemotherapy
11/54	AML	ROAP → CR; methyl-gag → F; AMSA → CR	22	800	Failure	No further chemotherapy

*AML = acute myelocytic leukemia; AMML = acute myelomonocytic leukemia; ALL = acute lymphocytic leukemia; APL = acute promyelocytic leukemia.

†AdOAP = doxorubicin, vincristine, cytarabine, and prednisone; CR = complete response; AIM = L-asparaginase, ifosfamide, and methotrexate; POMP = purinethol, vincristine, 6-mercaptopurine, and prednisone; CBV = high-dose cyclophosphamide, carmustine, and etoposide; ABMT = autologous bone marrow transplantation; PR = partial response; Pip TBI = piparazinedione and total-body irradiation; ROAP = zorubicin, vincristine, cytarabine, and prednisone; CROP = cyclophosphamide, zorubicin, vincristine, and prednisone; CHOP-bleo = cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin.

REFERENCES

1. AHMANN F, MEYSKENS F, JONES S, ET AL. A broad phase II trial of AMSA with in vitro stem cell culture drug sensitivity correlation. Proc Am Assoc Cancer Res and ASCO 21:369, 1980.
2. LEGHA SS, BLUMENSCHEIN GR, BUZDAR AU, ET AL. Phase II study of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (AMSA) in metastatic breast cancer. Cancer Treat Rep 63:1961–1964, 1979.
3. LEGHA SS, KEATING MJ, ZANDER AR, ET AL. 4′(9-Acridinylamino)methanesulfon-*m*-anisidide (AMSA): a new drug effective in the treatment of acute adult leukemia. Ann Intern Med 93:17–21, 1980.
4. CABANILLAS F, LEGHA SS, BODEY GP, ET AL. Initial experience with AMSA as single agent treatment against malignant lymphoproliferative disorders. Blood 57:614–616, 1981.
5. ZANDER AR, SPITZER G, LEGHA S, ET AL. High dose AMSA and autologous bone marrow transplantation (ABMT) in patients with solid tumors. Proc Am Assoc Cancer Res and ASCO 22:234, 1981.

m-AMSA in Disseminated Prostatic Carcinoma: A Phase II Study[1,2]

Anibal Drelichman,* David A. Decker, Muhyi Al-Sarraf, and C. B. Dhabuwala[3,4]

Prostatic carcinoma is a leading cause of morbidity and mortality from cancer in the elderly male population of the United States (1). Several chemotherapeutic agents have been explored for use in disseminated disease. Cyclophosphamide, 5-FU, cisplatin, and doxorubicin all demonstrated activity in phase II and III clinical trials (2–4). To determine the efficacy of *m*-AMSA, a new acridine derivative in the management of disseminated prostatic carcinoma, a phase II study was conducted at Wayne State University (5).

MATERIALS AND METHODS

Patients with histologically confirmed carcinoma of the prostate, regardless of previous treatment, were eligible. They were to receive no hormonal or cytotoxic therapy for > 4 weeks prior to entering the study. Before patients entered the study, extent of disease was documented by physical examination, bone scan, bone survey, pelvic computerized tomographic scan, and acid phosphatase. All patients were seen every 3 weeks, and lesions were measured. Pertinent tests were repeated as warranted during the course of therapy. Response was defined according to National Prostatic Cancer Project criteria (4).

Good-risk patients (≤ 65 years of age, wbc count ≥ 4000/mm^3, platelet count ≥ 100,000/mm^3, bilirubin ≤ 1.5 mg/100 ml, prior radiation therapy to < 49% of functioning bone marrow, and/or no prior chemotherapy) received 120 mg/m^2 of *m*-AMSA every 3 weeks. Poor-risk patients (> 66 years of age, wbc count of 2500–3999/mm^3, platelet count of 75,000–99,999/mm^3, bilirubin of 1.6–3.0 mg/100 ml, radiation therapy to ≥ 50% of functioning bone marrow, and/or intolerance to prior chemotherapy) received 100 mg/m^2 of *m*-AMSA every 3 weeks.

RESULTS AND DISCUSSION

Twenty-six patients were entered in this study. Their characteristics, previous therapy, and response are presented in table 1. All patients were evaluable for toxic effects. Five patients were not evaluable for response; four died < 3 weeks from beginning of therapy, and one had repeated biopsies of the prostate that failed to prove the primary site. Twenty-five of 26 patients were poor-risk.

No complete or partial remissions were observed. Ten patients (47.6%) had stable disease, with a median survival of 48 weeks. Of the patients with stable disease, one had a < 50% decrease in size of pelvic nodes, two had improvement of myelophthisic anemia, and one had improvement in bone scan. The remaining 11 patients (52.2%) developed progressive disease, with a median survival of 14.4 weeks (table 1).

Toxicity was mainly hematologic. Two patients died from sepsis secondary to myelosuppression. Leukopenia was observed in 23 patients (88.5%); thrombocytopenia was observed in 12 (46.2%). Mild nausea and vomiting occurred in two patients. Two patients had mild alopecia, and one had skin hyperpigmentation.

We conclude that *m*-AMSA in this dose and schedule did not produce complete or partial remissions in this poor-risk population. The dose-limiting toxic effect, leukopenia, was severe in these patients, with two drug-related deaths.

[1]Received Mar 16, 1982; revised Aug 3, 1982; accepted Aug 19, 1982.

[2]Supported in part by contract N01-CM-07404 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Oncology (*A. Drelichman, D. A. Decker,* and *M. Al-Sarraf*) and Urology (*C. B. Dhabuwala*), Wayne State University, School of Medicine, Detroit, MI.

[4]We thank Mary Beaker and Lynn McCloud for secretarial and data management support.

**Reprint requests to:* Anibal Drelichman, MD, Department of Oncology, Harper-Grace Hospitals, 3990 John R, Detroit, MI 48201.

TABLE 1.—*m*-AMSA in prostatic carcinoma: patient characteristics, response, and toxicity

	No. of patients (%)
Eligibility	
Total entered	26
Evaluable for toxic effects	26
Evaluable for response	21*
Age in yrs (n = 26)	
Mean: 67.3	
Range: 55–90	
Performance status (n = 26)	
Mean: 67.5	
Range: 40–90	
Predominant site of metastases (n = 26)	
Bone	23 (88.5)
Viscera	3 (11.5)
Previous therapy (n = 26)	
Orchiectomy	4 (14)
Additive hormonal	3 (12)
Orchiectomy and hormonal	19 (74)
Radiation†	16 (62)‡
Chemotherapy†	11 (43)‡
Response (n = 21)	
Complete/partial	0 (0)
Stable disease	10 (47.6)§
Progressive disease	11 (52.4)¶
Toxic effects (n = 26)	
Leukopenia	23 (88.5)
Mild/moderate	5 (19.3)
Severe/life-threatening	18 (69.2)
Thrombocytopenia	12 (46.2)
Mild/moderate	5 (19.2)
Severe/life-threatening	7 (26.9)
Nausea/vomiting	2 (7.0)
Skin hyperpigmentation	1 (3.0)
Alopecia	2 (7.0)

*5 patients inevaluable for response because of early death (4 patients) and adenocarcinoma of unconfirmed primary site (1 patient).
†In addition to hormonal therapy.
‡% of all cases. Some patients had both.
§Median survival: 48 wks.
¶Median survival: 14.4 wks.

REFERENCES

1. Cancer: Facts and Figures. American Cancer Society, 1980.
2. TORTI G, and CARTER SK. The chemotherapy of prostatic carcinoma. Ann Intern Med 92:681–689, 1980.
3. SCHMIDT J, SCOTT WW, GIBBONS R, ET AL. Chemotherapy programs of the National Prostatic Cancer Project (NPCP). Cancer 45:1937–1946, 1980.
4. SCHMIDT J, JOHNSON DE, SCOTT WW, ET AL. Chemotherapy of advanced prostatic cancer. Evaluation of response parameters. Urology 7:602–610, 1976.
5. VON HOFF DD, HOWSER D, GORMLEY P, ET AL. Phase I study of methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]-(*m*-AMSA) using a single-dose schedule. Cancer Treat Rep 62:1421–1426, 1978.

Phase II Trial of Mitoxantrone in Advanced Colorectal Cancer[1,2]

Eric M. Bonnem, Edith P. Mitchell, Paul V. Woolley,* Frederick P. Smith, John Neefe, Leroy Smith, and Philip S. Schein[3]

Mitoxantrone (DHAD) is an anthraquinone that has shown antitumor activity in several preclinical test systems. It exhibits less cardiotoxicity than doxorubicin in the rodent (1). Phase I studies of DHAD defined a tolerable dose of 8–12 mg/m^2 for phase II trials (2–5). Disease-oriented trials in acute leukemia (5) and breast carcinoma (6) have been reported. We report here a phase II study of DHAD in advanced colorectal carcinoma.

Between January and September 1981, 45 patients with advanced, histologically proven adenocarcinoma of the colon and rectum were treated with DHAD. Characteristics of this group are shown in table 1. Forty-one patients had received prior radiation therapy and/or chemotherapy, while four had not received prior therapy. Thirty-nine patients had been previously treated with some chemotherapy; all of these patients had received 5-FU, 47% had received nitrosoureas, 14% had received mitomycin, and several had received other investigational drugs. The median performance status was 1, and all patients had at least one site of objectively measurable disease. A partial response was defined as a $> 50\%$ reduction in all sites of measurable disease, while a complete response was defined as complete disappearance of all measurable disease, with no appearance of disease elsewhere. The liver was considered to be a measurable lesion if it contained proven metastatic disease and was enlarged at least 5 cm below the costal margin. In 22 of the previously treated patients, the initial dose of DHAD was 8 mg/m^2 every 3 weeks; three of these patients had subsequent dose escalation from 8 to 10 mg/m^2 when no myelotoxicity had been demonstrated at the lower dose. Twenty-one patients were treated at a dose of 10 mg/m^2 later in the study when it became apparent that the 8-mg/m^2 dose was well-tolerated. Two patients were treated at a dose of 12 mg/m^2. The number of courses per patient is shown in table 2.

None of the 45 patients had a clinical response to DHAD treatment. The major toxic effect was dose-related myelosuppression (table 2), which was reversible in all cases. In those patients who received multiple doses, there was little variation in successive nadir counts, suggesting the absence of cumulative myelotoxicity. All of the patients who received repeat drug doses were able to receive the subsequent dose of DHAD on schedule. One patient who had received a dose of 10 mg/m^2 became febrile, with a wbc count of 1.2×10^3/mm^3; blood cultures grew *Enterobacter spp* that responded to gentamicin, and the patient recovered. This patient had been previously treated with 5-FU and semustine.

Nonhematologic toxicity was mild. Slight nausea and vomiting occurred in three patients. There were two episodes of gastrointestinal bleeding; one was thought to be secondary to a necrotic malignant ulcer. Both episodes were terminal events and neither was related to thrombocytopenia.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	45
Males	22
Females	23
Median age in yrs (range): 61 (31–78)	
Prior therapy	
None	4
Chemotherapy	23
Radiation	2
Chemotherapy + radiation	16
Median performance status (range): 1 (0–2)	

[1]Received Apr 1, 1982; revised Aug 5, 1982; accepted Aug 19, 1982.
[2]Supported by contract N01-CM-97208 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]Division of Medical Oncology, Department of Medicine, Georgetown University Hospital and Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC.
*Reprint requests to: Paul V. Woolley, MD, Rm 414, Vincent T. Lombardi Cancer Research Center, 3800 Reservoir Rd, NW, Washington, DC 20007.

TABLE 2.—No. of courses of mitoxantrone and hematologic toxicity

	Dose (mg/m²)		
	8	10	12
	*No. of patients**		
No. of courses			
1	4	7	2
2	10	7	
3	5	5	
4	4	1	
	Hematologic toxicity†		
Median wbc count nadir, $\times 10^{-3}$ cells/mm³	3.3 (1.6–8.7)	3.4 (1.2–11.10)	1.5, 10.3‡
Median platelet count nadir, $\times 10^{-3}$ cells/mm³	193 (80–494)	248 (105–416)	230, 234‡

*Each entry specifies No. of patients who received indicated No. of courses at each dose level.

†Values in parentheses = range.

‡Only 2 patients.

There were no cases of congestive heart failure; none of the patients had received anthracyclines. In 13 patients, disease progression was documented after one course, in 17 patients after two courses, in ten patients after three courses, and in five patients after four courses of DHAD (table 2).

In view of subsequent experience with DHAD, the doses used in this study were somewhat low. Previously treated patients were started at a dose of 8 mg/m² because at the time the trial was begun, there was information to suggest that the dose-response curve for toxicity at doses > 10 mg/m² was steep and that 14 mg/m² would be the maximal tolerated dose. While the 8- and 10-mg/m² doses were well-tolerated, they have been associated with toxic effects; 30% of the patients treated at a dose of 8 mg/m² and 40% of those treated at a dose of 10 mg/m² had wbc count nadirs < 3×10^3/mm³. No indication of drug activity that would encourage use of higher doses was seen. In summary, on the basis of these data, DHAD has no activity in advanced colon carcinoma in this dose range and schedule.

REFERENCES

1. CHENG CC, ZBINDEN G, and FEE-CHENG RKY. Comparison of antineoplastic activity of aminoethylamino anthraquinones and anthracycline antibiotics. J Pharm Sci 68:393–396, 1979.
2. VON HOFF DD, POLLARD E, KUHN J, ET AL. Phase I clinical investigation of 1,4-dihydroxy-5,8-bis(((2-((2-hydroxyethyl)amino)ethyl) amino))-9,10-anthracenedione dihydrochloride (NSC 301739), a new anthracenedione. Cancer Res 40:1516–1518, 1980.
3. WYNERT W, HARVEY H, LIPTON A, ET AL. A phase I study of DHAD. Proc Am Assoc Cancer Res and ASCO 22:352, 1981.
4. UNGERLEIDER RS, COHEN LF, GLAUBIGER DL, ET AL. Dihydroxyanthracenedione: phase I evaluation in children. Proc Am Assoc Cancer Res and ASCO 22:392, 1981.
5. ESTEY EH, KEATING MJ, MCCREDIE KB, ET AL. Phase I–II study of dihydroxyanthracenedione in acute leukemia. Proc Am Assoc Cancer Res and ASCO 22:490, 1981.
6. YAP HY, BLUMENSCHEIN GR, and SCHELL FC. Phase II study of DHAD in metastatic breast cancer. Proc Am Assoc Cancer Res and ASCO 22:161, 1981.

Phase II Trial of Bisantrene in Advanced Colorectal Cancer: A Cancer and Leukemia Group B Study[1,2]

Michael C. Perry,[3,*] Arlene A. Forastiere,[4] Frederick Richards, II,[5] Raymond B. Weiss,[6] and Dan Anbar[7]

Bisantrene is a synthetic anthracenedicarboxaldehyde hydrazone with antitumor activity in a variety of animal systems including colon line 26 (1). Its mechanism of action is unclear but in vitro studies indicate that bisantrene, which contains a planar electron-rich chromophore, may act as a DNA intercalating agent. In phase I studies the dose-limiting toxic reaction was reversible leukopenia, but nausea and vomiting, local irritation, phlebitis, chills, fever, hypotension, and anaphylactoid reactions were also observed (2). The activity of bisantrene against human tumors is unknown. Since there are few chemotherapeutic agents effective against advanced colorectal cancer, the Cancer and Leukemia Group B (CALGB) elected to evaluate the drug in patients with this disease.

MATERIALS AND METHODS

Eligibility criteria for this study included histologic confirmation of unresectable or metastatic colorectal carcinoma, measurable tumor, a CALGB performance score ≤ 3, the absence of cardiac disease, and informed consent. Patients were required to have a wbc count $\geq 4000/\mu l$, platelet count $\geq 100{,}000/\mu l$, hemoglobin ≥ 10 g/dl, BUN ≤ 25 mg/dl, creatinine ≤ 1.5 mg/dl, and bilirubin ≤ 1.5 mg/dl. At least 4 weeks had to have elapsed from major surgery or radiotherapy. Our goal was to study patients not previously exposed to chemotherapy. However, we did not exclude patients who had received adjuvant chemotherapy > 1 year prior to the study.

Based on phase I observations, 260 mg/m^2 of bisantrene was administered iv over at least 60 minutes every 3 weeks. One dose escalation to 280 mg/m^2 was permitted, and the protocol provided for dose reductions based on nadir blood cell counts and those obtained on the day of treatment. Minimum treatment was three cycles (9 weeks) unless there was objective evidence of disease progression.

RESULTS

Thirty-nine patients were entered in this study. Seven were ineligible because of prior chemotherapy in the year preceding entry, leaving 32 patients. There were 22 males and ten females, with a median age of 61.7 years (range, 33–75). All patients had a CALGB performance score ≤ 2. Twenty-one patients had been treated with surgery alone. Eight patients had received prior adjuvant chemotherapy, radiotherapy, or both. Sites of measurable disease included liver (20 patients), lung (11), abdominal masses (five), lymph nodes (four), subcutaneous masses (three), and bone (three). All patients received the first course of therapy, 26 received a second, 17 a third, four a fourth, and one a fifth course. There were 17 dose escalations and two reductions.

No patient had a partial or complete response. Nine patients were stable, 20 had progressive disease, and there were three early deaths, all tumor-related.

Hematologic toxicity was mild, with median nadir wbc counts of $4700/\mu l$ (range, 1100–6900) and platelet counts of $277{,}000/\mu l$ (range, 100,000–544,000). The most striking toxic reactions were fever, chills, and phlebitis (table 1). Following treatment, 12 patients (38%) experienced fever and chills, occasionally with hypotension. Nine patients (28%) reported burning at the iv

[1]Received May 20, 1982; revised Aug 12, 1982; accepted Aug 19, 1982.
[2]Supported by Public Health Service grant CA-31946 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]University of Missouri Health Sciences Center, Columbia.
[4]Baltimore Cancer Research Center, University of Maryland.
[5]Bowman-Gray School of Medicine, Winston-Salem, NC.
[6]Walter Reed Army Medical Center, Washington, DC.
[7]Cancer and Leukemia Group B, Brookline, MA.
Reprint requests to: Michael C. Perry, MD, Department of Medicine, N408, University of Missouri Health Sciences Center, Columbia, MO 65212.

TABLE 1.—Patient characteristics

	No. of patients
Eligible for study	32
Sex	
Male	22
Female	10
Median age (range) in yrs: 61.7 (33–75)	
Performance status	
0	14
1	9
2	9
Toxicity	
Nonhematologic	
None	11 (34%)
Fever/chills	12 (38%)
Hypotension	2 (6%)
Nausea/vomiting (≥ grade 2)	3 (9%)
Phlebitis	9 (28%)
Hematologic*	
Median nadir wbc count × 10^3 (range): 4.7 (1.1–6.9)	
Median nadir platelet count × 10^3 (range): 277 (100–544)	

*Data are based on 32 courses given at a dose of 260 mg/m^2.

site or developed thrombophlebitis following the infusion. Nausea and vomiting of at least grade 2 (moderate) severity were mentioned by only three patients (9%).

DISCUSSION

This phase II trial failed to identify any activity of bisantrene in patients with recurrent or metastatic colorectal cancer who had not received previous chemotherapy for metastatic disease. Side effects were fever, chills, and phlebitis. At this dose and schedule bisantrene is inactive in this disease.

REFERENCES

1. CITARELLA RV, WALLACE RE, MURDOCK KC, ET AL. Activity of a novel anthracenyl bishydrazone, 9,10-anthracenedicarboxaldehyde bis [4,5-dihydro-1H-imidazol-2-yl) hydrazone] dihydrochloride, against experimental tumors in mice. Cancer Res 42:440–444, 1982.
2. VON HOFF DD, MYERS JW, KUHN J, ET AL. Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis [4,5-dihydro-1-imidazol-2-yl) hydrazone] dihydrochloride (CL 216,942). Cancer Res 41:3118–3121, 1981.

VAP-Cyclo: A Short Intensive Regimen of Chemotherapy for Advanced Breast Cancer

Combination chemotherapy will produce responses in 53%–84% of patients with metastatic breast cancer who have had no prior exposure to cytotoxic drugs (1). In addition, there is now evidence that adjuvant chemotherapy, given after mastectomy in patients who have involved axillary nodes, reduces the incidence of relapse in certain subgroups (2). Unfortunately, many regimens require frequent clinic visits for periods up to 1 year, although there are data supporting shorter courses of adjuvant chemotherapy (3–5). Toxic effects of therapy such as nausea, vomiting, and alopecia may be bearable for short periods but often become less tolerable with time, so the concept of a short intensive course of adjuvant chemotherapy, starting early in the postoperative period and being completed within 6 weeks, is attractive to both physician and patient. We report the results of a pilot study designed to test the efficiency and tolerance of such a combination in patients with metastatic disease.

TABLE 1.—Patient characteristics

No. of patients	
Premenopausal	10
Early postmenopausal (UICC)	2
Late postmenopausal	9
Median (range)—	
Age in years: 53 (31–64)	
Karnofsky performance status: 70% (50%–90%)	
Disease-free interval in mos: 33 (3–132)*	
Interval from 1st recurrence to chemotherapy in mos: 2 (0–27)*	
No. of patients with prior—	
Surgery only (mastectomy or biopsy)	11
Radiotherapy	5
Hormone therapy	
Tamoxifen	8†
Progestogen	2‡
Chemotherapy	0
No. of disease sites	
Primary tumor	6
Lung and pleura	14
Bone	13
Liver	5
Soft tissue	6
Nodes	11
Bone marrow	4
Brain	2

*Excluding 6 patients with unresected primary.
†2 responses lasting 14 and 15 mos.
‡No response.

Patient characteristics are shown in table 1. The dominant sites of disease were primary tumor (six patients), lung (seven), liver (three), soft tissue and nodes (four), and bone (one). Disease was present at two sites in ten patients and at three or more sites in eight patients.

Chemotherapy (VAP-Cyclo) consisted of 50 mg/m^2 of doxorubicin iv and 500 mg/m^2 of cyclophosphamide iv in alternate weeks × 3; 1.4 mg/m^2 of vincristine weekly × 6; and 25 mg/day of prednisolone orally continuously for 6 weeks. Patients were reassessed at 8 weeks, and further treatment depended on the response to the induction course. The majority of patients received further chemotherapy with 50 mg/m^2 of doxorubicin iv and 500 mg/m^2 of cyclophosphamide iv every 3 weeks (A/C).

Twenty-one of the 25 patients entered in the study were considered to be evaluable. Of the four inevaluable patients, one had no clear evidence of disease progression 1 month after oophorectomy, and the remaining three received inadequate chemotherapy. Reassessment 2 weeks after the completion of VAP-Cyclo showed three patients to be in complete remission and six to be in partial remission by International Union Against Cancer (UICC) criteria: the overall response rate was 42.8%. One of the patients who had complete remission had cerebral metastases controlled by irradiation and dexamethasone in addition to a pulmonary metastasis which disappeared. Disease stabilization was observed in eight patients, and three had disease progression. There was one early death (26 days) due to toxicity.

The 17 patients showing remission or disease stabilization received further chemotherapy with A/C; the number of courses ranged from one to ten. During this therapy, three more patients achieved complete remission; two of the three had shown minor response, and the other had had a partial remission at the end of VAP-Cyclo. The durations of response for patients with complete remission are 8, 10, 13, 14, 13+, and 14+ months. Five patients remained in partial remission for 7, 7, 8, 10, and 12 months; four had stable disease for 2, 3, 5, and 7 months; and two had disease progression. The overall response rate to VAP-Cyclo and A/C was 52.4%. Two complete remissions were in lung, one was in lung and liver, one was in soft tissue, and the remaining two were regressions of large primary tumors. The dominant sites of disease in the five patients with partial remission were liver, primary tumor, soft tissue, bone, and lung (one patient each).

Toxicity (21 Evaluable Patients)

Dose modification.—Vincristine was omitted once in four patients and four times in one patient, usually because of mild weakness or severe constipation. Myelosuppression necessitated a delay of 1 week on one occasion in four patients and a delay of 1 week on two occasions in two patients.

Hematologic.—The mean wbc count nadir was 2.6×10^9/L (range, 0.6–6.2), with only three patients having a nadir $< 1.0 \times 10^9$/L. The mean platelet count nadir was 195×10^9/L (range, 25–216), with two patients having a nadir $< 50 \times 10^9$/L. Four patients with myelosuppression were known to have bone marrow infiltration. There were two significant infections requiring iv antibiotic treatment. One patient who had extensive liver, lung, and pulmonary metastases died after 26 days from septicemia and bleeding secondary to myelosuppression (wbc count nadir, 0.6×10^9/L; platelet count nadir, 50×10^9/L).

Nonhematologic.—Nausea and vomiting of varying severity occurred in all patients, but treatment modification was not required. Oral candidiasis occurred in seven patients and alopecia was universal. Significant peripheral neuropathy occurred in only one patient, although paresthesias in the extremities were common.

This study has shown that an intensive 6-week induction course of chemotherapy with VAP-Cyclo is active against metastatic breast cancer. The majority of patients in this study had a substantial tumor burden that would be unlikely to resolve completely after a 6-week induction course of chemotherapy. However, in the adjuvant situation, complete destruction of the systemic micrometastases of responsive tumors might be possible in a 6-week period, although this remains to be tested.

Combination VAP-Cyclo was generally well-tolerated and toxic effects were usually rapidly reversible. In fit patients with good hepatic and renal functions and normal bone marrow, this particular combination would probably be well-tolerated and would be acceptable as an adjuvant treatment if patients were carefully monitored during the 8-week, high-risk period.

REFERENCES

1. Rubens RD. Breast cancer. *In* EORTC Annual 3 (Pinedo HM, ed). Excerpta Medica, 1981, chap 19, pp 360–396.
2. Bonadonna G, Rossi A, Valagussa P, et al. The CMF programme for operable breast cancer with positive axillary nodes: updated analysis on the disease free interval, site of relapse and drug tolerance. Cancer 39:2904–2915, 1977.
3. Tancini G, Bajetta E, and Marchini S. Preliminary 3 yr results of 12 versus 6 cycles of surgical adjuvant CMF in premenopausal breast cancer. Cancer Clin Trials 2:285–292, 1979.
4. Nissen-Meyer R, Kjelleren K, Malmio K, et al. Surgical adjuvant chemotherapy: results with one short course with cyclophosphamide after mastectomy for breast cancer. Cancer 41:2088–2098, 1978.
5. Henderson IC, Gelman R, Parker LM, et al. 15 vs. 30 weeks (wks) of adjuvant chemotherapy for breast cancer patients (pts) with a high risk of recurrence: a randomised trial. Proc ASCO 1:75, 1982.

Vivien H. C. Bramwell
A. Howell
H. Scarffe
D. Crowther

Cancer Research Campaign
Department of Medical Oncology
Manchester University
Christie Hospital and Holt Radium Institute
Wilmslow Rd
Manchester M20 9BX
England

R. A. Sellwood

Department of Surgery
Withington Hospital
Nell Lane
Manchester M20 9BX
England

July 30, 1982

Subcutaneous Reaction Due to Intra-arterial Infusion of Doxorubicin

Intra-arterial infusion of doxorubicin is often used in the chemotherapeutic protocol for the treatment of some tumors (1). The toxic effects of doxorubicin include myelosuppression, cardiotoxicity, gastrointestinal toxicity, alopecia, and stomatitis (1–2). In addition, extravasation of doxorubicin may cause skin necrosis and ulcers (3). Skin discoloration occurring during intra-arterial infusion of doxorubicin and extending over the area of an osteosarcoma has not yet, to our knowledge, been described.

CASE REPORT

A $12^1/_2$-year-old girl was admitted to the hospital because of constant pain in her right knee. A biopsy established the diagnosis of an osteogenic sarcoma. Our treatment protocol included three courses of doxorubicin (60 mg/m^2) and cisplatin (100 mg/m^2) prior to surgery. While the patient was receiving the doxorubicin (100 mg in 100 ml of solution), a large (10 × 15 cm) brownish-purple area of discoloration was noted on the

extensor surface of the right thigh. The infusion was discontinued. Extravasation was excluded by demonstrating angiographically that the tip of the catheter was in the femoral artery. The lesion was not associated with any neurologic impairment. A skin biopsy 1 month later showed intracellular and intercellular edema of the epidermis with pyknosis of the nuclei. Telangiectatic capillaries were present just below the flattened epidermis. This area of pigmentation has faded and decreased in size but has not completely disappeared even after 3 years.

DISCUSSION

To achieve high concentrations of cytotoxic drugs in limited anatomic areas occupied by tumor, regional chemotherapy is given by intra-arterial infusion. This method of treatment enables us to confine the drug to the involved area and therefore reduces the systemic side effects. In our patient we intended to perform limb salvage as the orthopedic procedure.

As no metastases were found, the goal of treatment was to decrease the tumor bulk before surgery. Doxorubicin was injected in the usual concentration into the femoral artery as verified by angiography. Haskell et al (4) described skin necrosis that developed 1 week after intra-arterial infusion of doxorubicin in a patient with esophagopharyngeal carcinoma. However, it occurred in an area that had previously received > 7000 rads of radiation therapy. Di Pietro et al (5) have described local reactions to intra-arterial infusion of doxorubicin, including thrombosis, occlusion, arteritis, necrosis of the skin, and neuritis, none of which were observed in our patient. Considering that neither malignant metastatic skin invasion nor skin necrosis was noted in this case, we presume that the high local concentration of doxorubicin per se was responsible for the local discoloration.

REFERENCES

1. Haskell CM, Eilber FR, and Morton DL. Adriamycin (NSC-123127) by arterial infusion. Cancer Chemother Rep Part 3, 6(2):187–189, 1975.
2. Rudolph R, Stein RS, and Pattillo RA. Skin ulcers due to adriamycin. Cancer 38:1087–1094, 1976.
3. Rudolph R, Suzuki M, and Luce JK. Experimental skin necrosis produced by adriamycin. Cancer Treat Rep 63:529–537, 1979.
4. Haskell CM, Silverstein JJ, and Rangel DM. Multimodality cancer therapy in men; a pilot study of adriamycin by arterial infusion. Cancer 33:1485–1490, 1974.
5. Di Pietro S, De Palo GM, and Gennari L. Cancer chemotherapy by intra-arterial infusion with adriamycin. J Surg Oncol 5:421–430, 1973.

A. Ballin
G. Kende
B. Morag
M. Ronen

Department of Pediatric Hematology
Chaim Sheba Medical Center
Tel-Hashomer
Israel

August 9, 1982

ANNOUNCEMENTS

Topics in Emergency Medicine

San Francisco, California, January 31–February 4, 1983

This course will be presented by Extended Programs in Medical Education, University of California School of Medicine, San Francisco and will take place at the Sheraton Hotel at Fisherman's Wharf. The program is designed to instruct emergency medical practitioners in depth through didactic presentations, case studies, student/teacher interactions, and demonstrations in the approach to problems in the emergency department. Upon completion of this program, registrants should be more competent in handling injuries of the hand and face, in diagnosing and treating acute cardiac disease, in interpreting radiographs, and in managing problems in other areas including pediatrics, ophthalmology, neurology, obstetrics/gynecology, and endocrinology. Pre and post course assessments will be available, as well as a syllabus with references.

This course meets the criteria for 35 hours of credit in the following: Category 1 of the AMA Physician's Recognition Award, the CMA Certification Program, the American Academy of Family Physicians, and Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, Rm 569-U, Third and Parnassus Ave, San Francisco, CA 94143 (telephone 415-666-4251).

Frontiers in Hematology/Oncology II: Complications of Neoplastic Disease in Therapy

Warren, Vermont, February 7–11, 1983

This meeting will take place at the Sugarbush Inn, Sugarbush Valley Ski Resort and will be presented in nine 2-hour sessions which will cover major topics of clinical interest to academic and practical hematologists, oncologists, and internists with special interest in hematology/oncology. Emphasis will be placed on review of the latest developments regarding the complications of treating patients with neoplastic diseases, pain control, infections in the immunocompromised host, newer antibiotics, and control of chemotherapy-induced toxicities. Credit for 20 hours of CME will be given. Registration fee is $275.

For more information, contact: Ms. Linda Bonacquisti, Albany Medical College, Division of Oncology, Albany, NY 12208 (telephone 518-445-5361).

Second Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 12–19, 1983

This symposium will feature a multidisciplinary faculty from the University of Arizona Cancer Center. They will review recent diagnostic and therapeutic advances in the lymphomas, leukemias, and multiple myeloma.

For further information, contact: Stephen E. Jones, MD, Section of Hematology and Medical Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Seventh Annual Cell Kinetics Society Meeting

Baltimore, Maryland, March 16–19, 1983

The seventh annual meeting of the Cell Kinetics Society will be held at Baltimore's Hilton Hotel. The Cell Kinetics Society has an international membership of approximately 300 experimentalists, clinicians, and mathematical modelers who share an interest in the cell proliferation patterns of normal and abnormal tissues.

For more information concerning this meeting or membership, contact: Bruce F. Kimler, PhD, Secretary, Cell Kinetics Society, Department of Radiation Therapy, University of Kansas Medical Center, Rainbow Blvd at 39th St, Kansas City, KS 66103.

Neuro-oncology Fellowships

University of California, San Francisco, July 1983

Two neuro-oncology fellowships will be available in the Department of Neurological Surgery/ Neuro-oncology Service at the University of California in San Francisco beginning in July 1983. The fellowships emphasize clinical and research training in primary adult and childhood tumors of the central nervous system. The first year is clinical and the second year is clinical or laboratory research. Candidates should be graduates of approved neurology or neurosurgical training programs. The University of California is an equal opportunity/affirmative action employer. Women and minorities are encouraged to apply.

For further information, contact: Dr. Victor A. Levin, Brain Tumor Research Center, 783 HSW, University of California Medical School, San Francisco, CA 94143.

Cancer Immunology Fellowship

Paris, France

A 2-year fellowship in clinical and laboratory cancer immunology is available at the Laboratoire Universitaire d'Immunobiologie des Tumeurs Humaines, and Chaire de Cancérologie. The program involves intensive training in medical oncology and clinical and laboratory work on human tumors, draining lymph nodes, thymus, spleen, and peripheral blood. Previous experience with monoclonal antibodies and/or HPLC techniques is preferable. Ability to communicate in French is necessary. Funds available are $1000 per month.

Candidates should send a curriculum vitae and an introductory letter from their laboratory director to: Professeur Lucien Israël, Hôpital Avicenne, Bobigny, 93000 France.

☆U.S. GOVERNMENT PRINTING OFFICE: 1982 361-140/5 1-3

DATE DUE

FEB 1 0 1988

GAYLORD

PRINTED IN U.S.A.

RIECKER MEMORIAL LIBRARY
ST. JOSEPH MERCY HOSPITAL
ANN ARBOR, MI 48106

a publication
of the
national cancer institute

NIH Publication No. 83-22